Journal of
DAIRY SCIENCE

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ANNUAL A.D.S.A. MEETING

North Carolina State College, Raleigh

June 16–19, 1958

JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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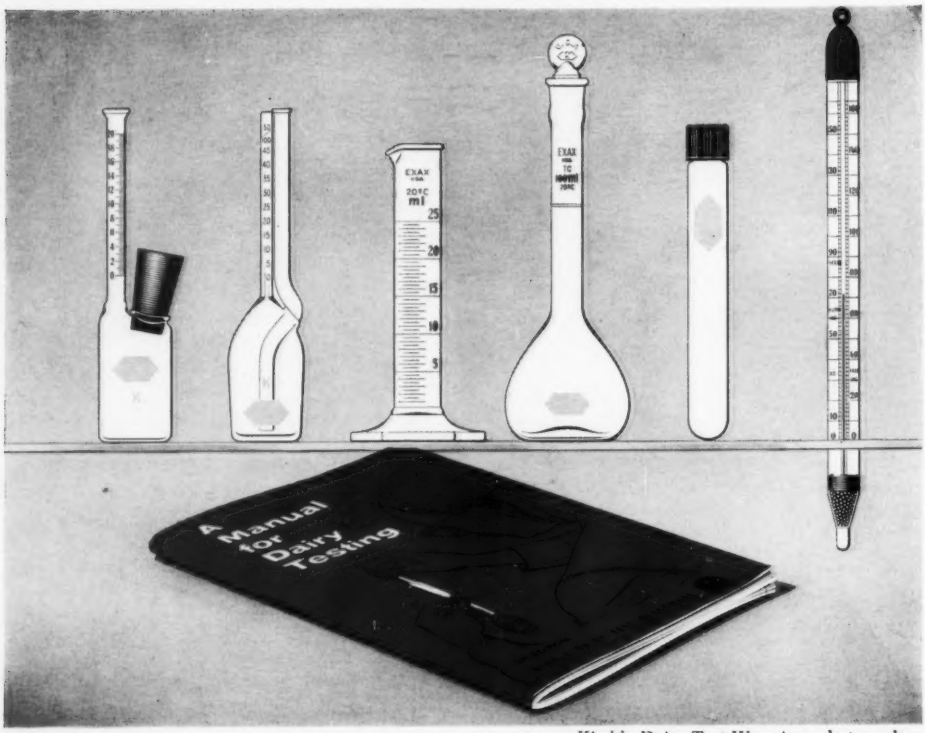

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PEOPLE AND EVENTS

F. W. Atkeson Dies

F. W. ATKESON, 64, head of the Dept. of Dairy Husbandry, Kansas State College, Manhattan, died April 4, 1958, at Riley County Hospital.

Mr. Atkeson was injured in an automobile accident in September of 1957 and had been in failing health since. He had not been in his office since early February.

A native of Butler, Mo., he received his B.S. in agriculture from the Univ. of Missouri, Columbia, in 1918 and his M.S. from Kansas State College in 1929. He was an assistant in the Kansas State Dairy Dept. from 1918 to 1920, a field representative for the American Jersey Cattle Club from 1920 to 1921, and professor of dairy husbandry and head of the department at the Univ. of Idaho, Moscow, from 1921 to 1935. In 1935 he returned to Kansas State as head of the Dairy Dept.

Mr. Atkeson was honored recently by friends in the feed industry, who established the Atkeson Loan Fund to recognize his work as chairman of the Kansas Formula Feed Conference for many years. He was also cited earlier this year by the Kansas Inter-Breed Dairy Cattle Council as a "Kansas Dairy Leader." He became the sixth Kansan to be so honored.

Mr. Atkeson judged many of the nation's top dairy shows and had been an official classifier for three dairy breeds. He was author or co-author of more than 100 publications dealing with dairying. In addition to being a member of the A.D.S.A., he was a member of the Society of Animal Production and the American Genetics Association.

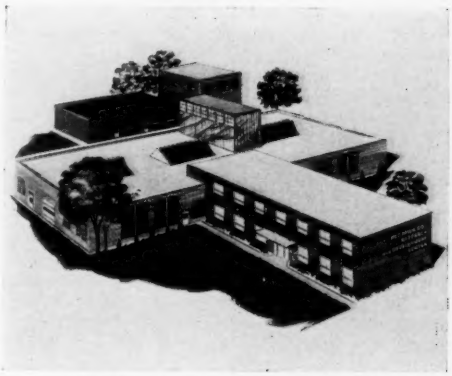
Survivors include his widow, Ferol; a son, George, U.S. Air Force, Alaska; two brothers, Ralph Wendell, Jefferson City, Mo., and Clarence E., Wichita, Kan., and a sister, Mrs. J. W. McCreery, Savannah, Ga.

Pet Milk Research Center Under Way

Construction work has started on expansion of the Pet Milk Company's Research and Development Center in Greenville, Ill., according to an announcement made in St. Louis by T. R. GAMBLE, executive vice-president of the Pet Milk Co.

The company plans to reorganize and expand its research staff and to undertake a broadened research program during 1958. Pet maintains extensive research facilities in Greenville and in its headquarters in St. Louis. Research activities in St. Louis will be continued and will be coordinated with those of the new Research and Development Center in Greenville.

Gamble said he expects the Pet research and development staff to be doubled within a short period of time. The entire research structure is being reorganized on a divisional basis.



The Pet Milk Company Research and Development Center now under construction at Greenville, Ill.

The eight major divisions of the structure will be nutrition, bacteriology, basic chemistry, dehydrated products, evaporated milk, ice cream and frozen dairy products, frozen prepared foods, and engineering design.

The new Research and Development Center will house several pilot plant operations, which will provide the intermediate steps between laboratory development and actual production. Each research unit or division dealing with a product category will have its own pilot plant equipment, adequate in size and scope to develop techniques for commercial production.

Heart of the expanded Center will be a new building adjoining the present pilot plant building. This new structure will be of brick and blue porcelain paneling, harmonizing with the two existing structures. Major laboratories and offices will be housed in the new building, which will feature the module arrangement of laboratories, complete air-conditioning, sound-proofing, and fluorescent lighting incorporated in fiber-glass panels.

The Center will incorporate some 30,000 square feet of space. In addition to the new structure, it will include the existing laboratory and the pilot plant building. The entire Center will be landscaped and will include a visitors' parking area.

Harmon Receives Point Four Citation

E. M. HARMON, senior livestock advisor to the Iranian Government under the U.S. Point Four program, received a citation of merit for some five years' work in the improvement of Iranian livestock production.

The citation read, in part, ". . . By your efforts and guidance your accomplishments have reached the grass roots of Iran and for these accomplishments you can be justifiably proud. You have well earned the respect and admiration of your colleagues and the appreciation of the Mission. . . ."

Mr. Harmon, a member of the A.D.S.A. for about 38 years, was chairman of the Extension Section of the Association from 1921 to 1923. He was also a member of the committee that arranged for bringing extension workers into the A.D.S.A.

Pou to Head Arizona Extension Work

J. W. Pou, head of the Dept. of Animal Industry, North Carolina State College, Raleigh, has been named director of the Arizona Cooperative Extension Program in Agriculture and Home Economics.

Dr. Pou will assume his new duties at the Univ. of Arizona, Tucson, on July 1.

Nebraska News

The Dairy Manufacturers Short Course was held in Omaha ending April 3. The course consisted of 12 sessions meeting once a week in the evenings. More than 40 people attended the classes.

Teaching most of the sessions were L. K. Crowe, P. A. Downs, K. M. Shahani, K. M. Nilson, T. A. Evans, and P. L. Kelly, all of the Dept of Dairy Husbandry, Univ. of Nebraska, Lincoln.

The Nebraska State Dairymen's Association meeting was held March 11. Guest speakers were W. E. Petersen, Univ. of Minnesota, St. Paul, and O. Olson, South Dakota State College, College Station.

The Nebraska Butter Institute was held March 18 on the Univ. of Nebraska campus, Lincoln. Guest speakers were R. L. Hruska, U. S. Senator from Nebraska; J. Anderson, Food and Drug Administration of the USDA, and V. H. Neilsen, Dairy Industry Dept., Iowa State College, Ames.

Thesis Completed

M.S. Degree

R. Nelson—A study of the various diluters for bovine semen. Univ. of Nebraska, Lincoln.

Herzer Named "Prof of Year" at Mississippi

F. H. Herzer, head of the Dept. of Dairy Husbandry, Mississippi State College, State College, was named "Professor of the Year"

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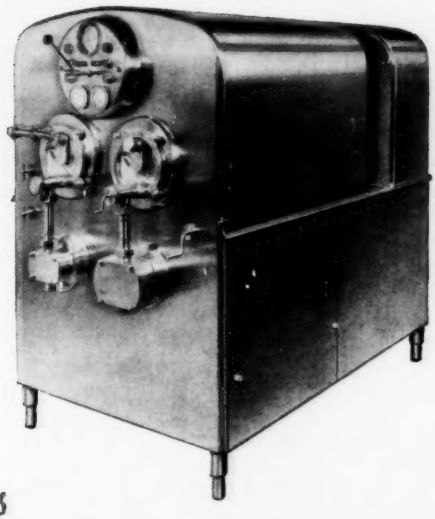

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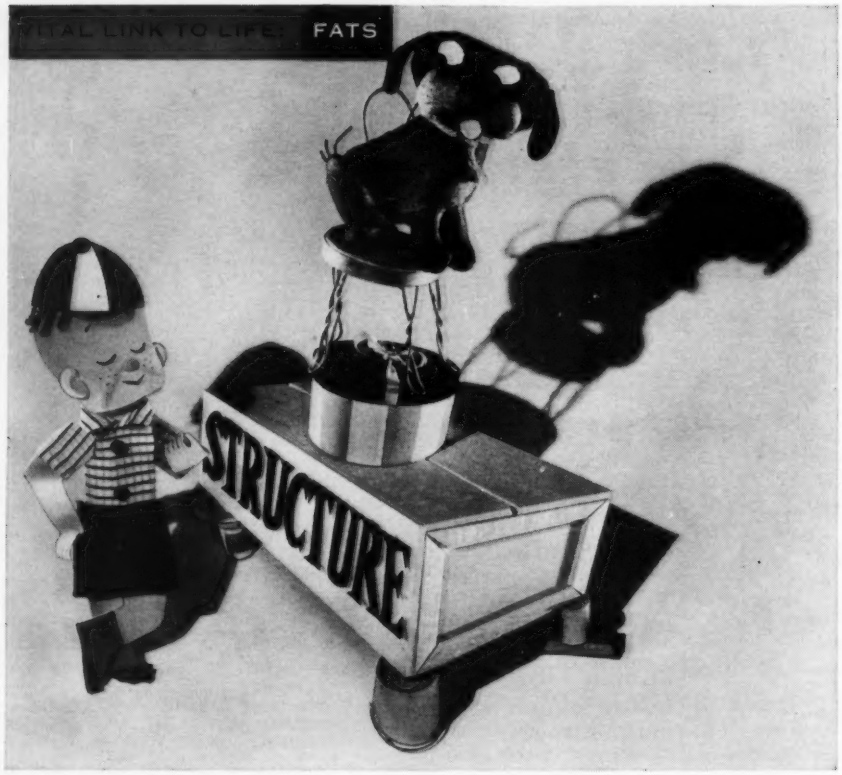

Fats contain a wide variety of fatty acids. Certain of these fatty acids are called "essential" . . . essential to the body for functioning in some vital and specific role . . . essential in foods because the body cannot synthesize them.

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at Mississippi State. Herzer, who has been on the Mississippi staff since 1919, has received numerous honors from dairy groups in the state.

A native of Marion, O., he received his B.S. degree in dairy technology from Ohio State Univ., Columbus, and his M.S. degree in dairy bacteriology from Iowa State College, Ames.

New Hampshire News

Construction has begun on the Nutrition Laboratory of the Dept. of Dairy Science, Univ. of New Hampshire, Durham. The new building will replace the one lost by fire in the latter part of 1956.

The new building will have facilities to work with a much larger number of animals than was possible in the old building. The laboratory will have two respiration chambers for cows and steers, as well as other chambers of various sizes to accommodate different species of animals.

The new building will also provide laboratory and office space for most of the research unit of the Dairy Science Dept.

A.D.A. Committee Meets

The Research-Education Committee of the American Dairy Association held its annual meeting March 18-19 in Chicago. Committee chairman was W. E. WINN and the research director was D. J. JACOBSEN.

The principal speakers were C. A. ELVEHJEM, R. T. MILNER, and J. R. MATCHETT. The invited guests spoke to the Research Committee members who were in session to pass on research proposals currently under consideration for American Dairy Association grants.

The A.D.A. is currently providing grants for 26 projects in 16 states. Six project renewals and one new project were approved in this meeting. The guest speakers discussed future objectives in dairy product and nutrition research.

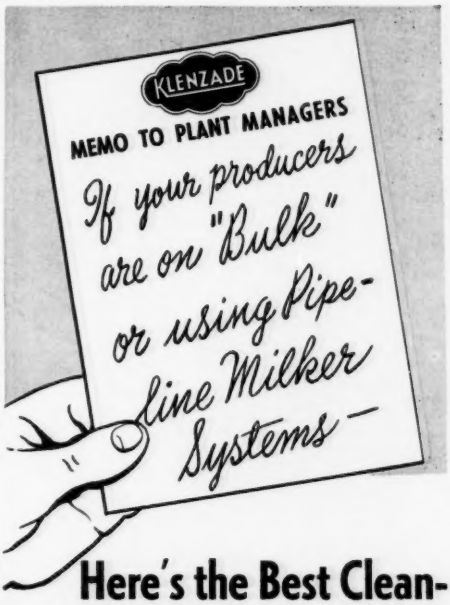
Scheinberg Joins USDA

S. L. SCHEINBERG, a geneticist from the Univ. of Wisconsin, Madison, has joined the Pioneering Research Laboratory, Animal Husbandry Research Division, ARS, USDA, Beltsville, Md. He will organize the new laboratory, where basic research on blood antigens and their relation to genetics and livestock production will be studied.

Dr. Scheinberg received his Ph.D. degree from Wisconsin, where he studied in the blood antigen laboratory under the supervision of R. M. ERWIN.

Florida News

The Florida Association of Milk and Food Sanitarians held its annual meeting and conference March 26-27 on the Univ. of Florida campus, Gainesville.



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More than 100 persons attended the meeting, which was under the general direction of W. A. KRIENKE, Univ. of Florida. D. L. LICHTY, Public Health Veterinarian, West Palm Beach, and president of the Association, conducted the business meeting. M. A. BROOKER, Dean of the Univ. of Florida College of Agriculture, welcomed the group.

Other participants in the program were R. J. MOONEY, Wyandotte Chemicals Corp., Wyandotte, Mich.; M. W. EMMEL, veterinarian for the Florida Agricultural Experiment Station; J. D. DENNIS, Dairy Division, Florida Dept. of Agriculture; R. B. BECKER, Dept. of Dairy Science, Univ. of Florida; B. J. LISKA, Dairy Science Dept., Univ. of Florida; J. E. MISTARZ, Chicago Stainless Equipment Corp.; S. NOLES, Florida State Board of Health; M. B. YOUNG, Florida Agricultural Extension Service; E. L. FOUTS, A. SHAW, M. GELBMAN, L. Z. SZABO, L. E. MULL, and L. L. CHAFFEE, all of the Univ. of Florida.

Association officers elected for 1958-59 were J. MASSEY, Pensacola, president; J. D. ROBINSON, Plant City, vice-president; B. NORTHRUP, St. Petersburg, secretary-treasurer, and the board of directors consisting of H. BROWN, Jacksonville; A. GRAHAM, Winter Haven; P. GRIFFIN, Tampa; H. JORDAN, Miami, and W. A. KRIENKE, Gainesville.

A Laboratory Technicians Short Course was held March 25-26 on the Univ. of Florida campus. J. TYLER, Volusia County Health Dept. Laboratories, was elected chairman of the group for the coming year. He succeeds Miss R. Vrooman, Velda Corp., North Miami Beach.

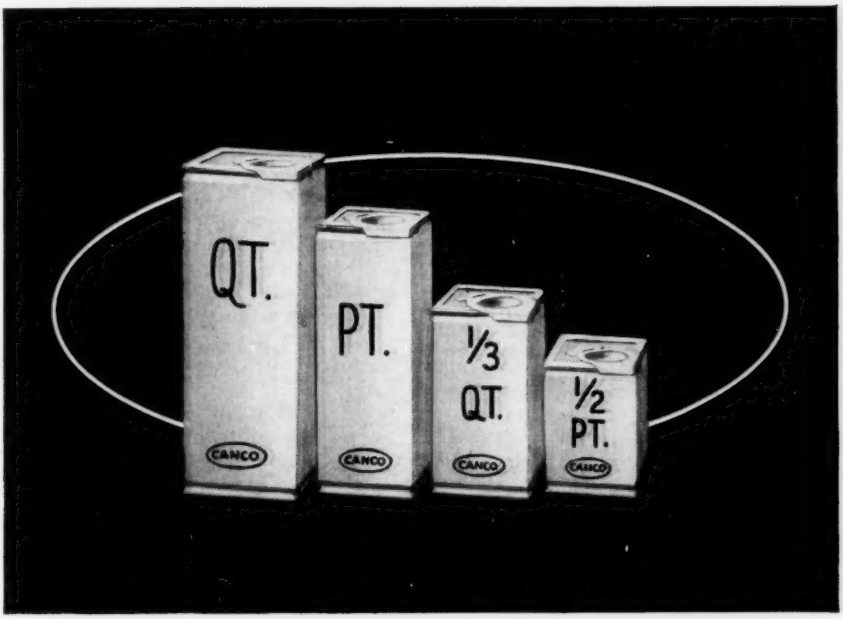
Other participants in the short course were B. J. LISKA and H. YOUNG, both of the Univ. of Florida, and H. BUTNER, Florida State Board of Health.

Louisiana News

Forty-five people from Louisiana and neighboring states registered for the two-day Dairy Products Quality Control Short Course held at the Louisiana State University, Baton Rouge, March 13–14. A. J. GELPI was general chairman. Speakers on the program were chosen from the LSU faculty, the dairy industry, related industry, and the Louisiana State Department of Health.

Because bulk tanks have but lately come to the South, bulk-tank handling and sanitation were given particular attention during the session. Another problem which farmers sometimes face in the Gulf Region is the low solids content of milk. L. L. RUSOFF explained how breeding, underfeeding, and the high Louisiana standards for milk composition were all related to this question.

On the second day a luncheon, sponsored by the local Dairy Products plants, was served at the Faculty Club.



MILK

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Many favorable comments were offered and requests for another Short Course next year were made by those who attended.

A yearly scholarship to be awarded to outstanding young dairymen of Louisiana has been established by the dairy industry of the State. J. M. CADWALLADER, one-time teacher of dairying at LSU and now owner of the Louisiana Creamery, as well as others interested in dairy promotion, has long felt that youngsters in the dairy business have not received the recognition given those who grow calves, pigs, or lambs, but it was not until this year that a unified program got under way to remedy this situation.

J. HIGHTOWER, 17-year-old 4-H Club member of Homer, has the honor of being the first one to receive the $1,000 scholarship by the Dairy Industry Scholarship Foundation. The scholarship provides $250 per college year for a period of four years, to the state school of his choice.

The purpose of the scholarship to to promote greater interest in junior dairy work. The winner must have a four-year over-all dairy record, including such specifications as junior dairy production records, dairy management, pasture management, cost of milk produced, and premiums won at various shows.

News from Connecticut

Two hundred people attended a Vacuum Flavor Removal Symposium held April 2, at the Univ. of Connecticut, Storrs.

W. A. COWAN, Head of the Animal Industries Dept., opened the morning session with a statement of the problem. He was followed by H. BEHLMER, Cherry-Burrell, D. GILLESPIE, Creamery Package, and W. D. JENKINS, DeLaval, who described the various types of vacuum flavor removal equipment marketed by their companies.

H. B. ROBINSON, Chief, Milk Sanitation Section, United States Public Health Service, described his agency's viewpoint concerning vacuum flavor removal equipment.

A. C. SMITH, Dairy Manufacturing Dept., Univ. of Connecticut, then presented a progress report on flavor removal research at the University of Connecticut.

A panel for questions and answers, consisting of L. R. GLAZIER, Dairy Manufacturing Dept., Univ. of Connecticut, and all other speakers, ended the session.

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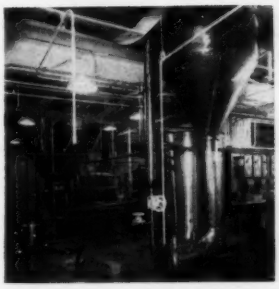

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Montana News

The second meeting of the Montana State College Dairy Industry Advisory Committee was held April 4, 1958.

This committee is made up of representatives of the milk processors and milk producers in the state and serves in an advisory capacity to the Dairy Industry Department.

Teaching, student enrollment, research, and extension problems were discussed at the last meeting.

Bausch & Lomb and Applied Research Labs Consolidate

C. S. HALLAUER, president of Bausch and Lomb Optical Co., Rochester, N. Y., and M. F. HASLER, president of Applied Research Laboratories, Glendale, Calif., have announced a consolidation of the two companies.

Under the new arrangement, the Applied Research Laboratories will be operated as a wholly owned subsidiary known as Applied Research Laboratories, Inc., a Subsidiary of Bausch and Lomb Optical Co.

Ohio State News

Some 150 high school students and industry representatives attended the Fifth Annual Dairy Technology Career Day at the Ohio State Univ., Columbus, April 12.

The event constituted the climax of the year's high school counseling and recruitment program which is conducted cooperatively by the organized dairy industry groups of Ohio and the Dept. of Dairy Technology at O.S.U.

The program included conducted tours of the research and teaching facilities of the department and addresses by students and faculty on the scholarship program, the curriculum, and the opportunities in the dairy technology field. Principal speakers included T. S. SUTTON, Associate Dean of the O.S.U. College of Agriculture; J. T. MOUNT, administrative assistant to the president of Ohio State, and J. H. ERB, vice-president of Borden's Midwest Division.

Massachusetts News

The Fourth Bulk Tank Truck Drivers' Workshop was held March 25–April 2 at the Waltham Field Station of the Univ. of Massachusetts. Material covered in the training session included composition and properties of milk, milk sampling, composite sample, pickup procedures, milk flavors, sanitation, and cleaning bulk tanks.

Most of the sessions were conducted by D. J. HANKINSON, D. EVANS, and J. BRAGG. During

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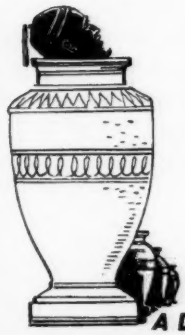

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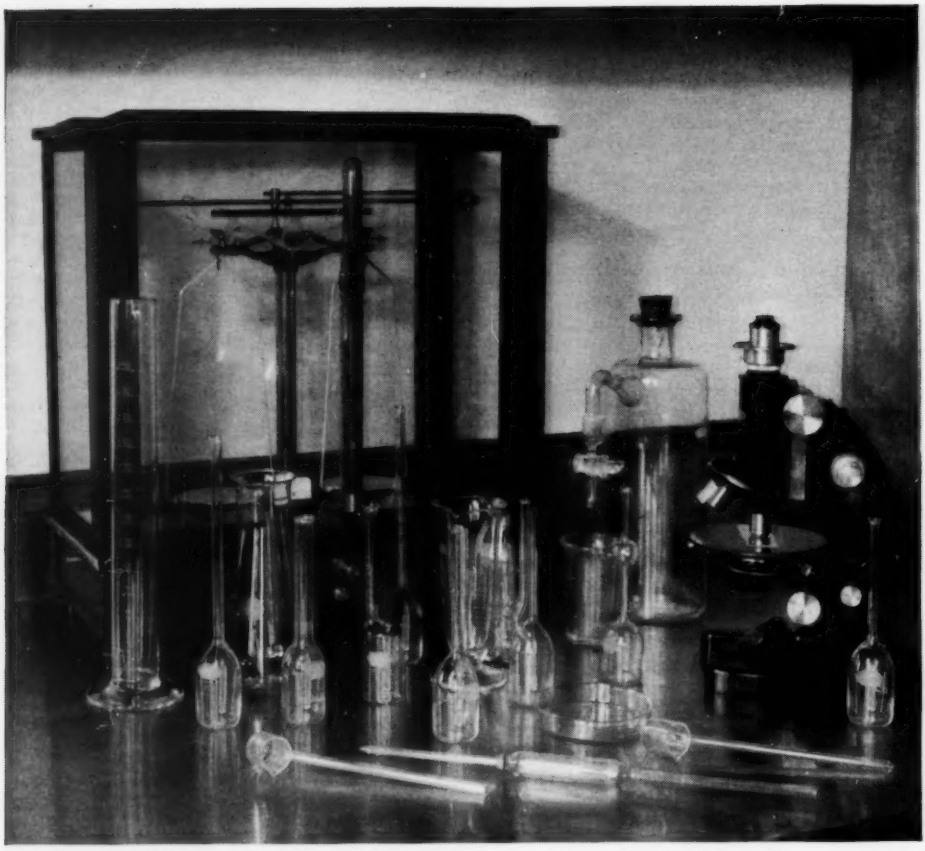

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the past two years, 116 groups have attended these evening training sessions. A breakdown of the enrollment shows 28 independent haulers and 88 persons representing 24 different dairies.

A Cottage Cheese Clinic was held April 9–10 in the Flint Laboratory of the Univ. of Massachusetts, Amherst. Fourteen industry members attended the clinic. Participants were N. C. ANGEVINE, Meyer-Blanke Co.; E. LUNDSTEDT, H. P. Hood and Sons; K. CORRIDAN, Sealtest Co., and D. EVANS, Dairy Dept., Univ. of Massachusetts.

Clemson News

The 1958 Annual Meeting of the South Carolina Federated Cooperative Breeding Association was held March 28 at Columbia, S. C. Featured speakers were H. A. HERMAN, executive secretary of the National Association of Artificial Breeders, and M. DRAKE, manager, N.O.B.A. Other participants were S. B. ROSE, president of N.O.B.A., and V. HURST, Clemson Dairy Dept.

C. G. CUSHMAN, Dairy Extension Dept., Clemson College, delivered the principal address at the 1958 N.O.B.A. meeting held March 22 in Tiffin, O. He discussed the artificial breeding program in South Carolina.

J. P. LA MASTER, former head of the Dairy Dept. at Clemson, has been appointed to serve the U.S. International Cooperation Administration in Brazil. He succeeds R. H. RUSH, who will return to his post at the Univ. of Tennessee, Knoxville.

The South Carolina Dairy Association, in cooperation with the Clemson College Dairy Dept., has established a technical subcommittee to combat present industry problems and to spot future ones. The subcommittee is working in seven selected areas, including efficiency of milk production, producer utilization, merchandising, distribution, and processing efficiency.

South Carolina had the first year-round surplus of milk needed for fluid consumption. As a result, changes affecting producers and processing may be expected in the industry almost immediately.

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STUDENT CHAPTER NEWS

Los Lecheros Dairy Club Honors

GEORGE DRUMM

George M. Drumm, retiring after a quarter century as head of California State Polytechnic College's dairy department, was honored by his former students as the eleventh annual Los Lecheros dairy majors' club banquet was turned over to commemoration of his many years of service and contribution to the college.

Drumm received from Robert Thomas, Fortuna, Los Lecheros president, an honorary membership certificate in the dairy majors' club. Club members presented a semiserious, semihumorous skit portraying Drumm's life as a student, teacher, and dairyman. Master of ceremonies for the evening was Weslie Combs, a former student of Drumm's, now head of the animal husbandry department at Cal Poly's Kellogg-Voorhis campus.

Also honored were outstanding seniors and the outstanding student dairy cattle judge of the department. Receiving the Golden State Trophy for outstanding dairy judge was Kenneth Winters, Wapato, Washington.

Gary Gilman of Fullerton, California, Ray Kliewer of Bellingham, Washington, and Robert Thomas of Fortuna, California, were awarded the George M. Drumm perpetual trophy as the three outstanding seniors.

James Merson, head of the Cal Poly agricultural engineering department, presented to Drumm a set of golf clubs, balls, tees, and a red plaid golf hat on behalf of the faculty and staff of the college. Ray Kliewer presented an engraved clock on behalf of the dairy students, alumni, and friends.

Three honorary Los Lecheros members were among the 165 in attendance: J. H. Sawyer, Galt, E. E. Greenough, Modesto, and James Emerson, San Luis Obispo.

Among now-prominent dairy department alumni who attended were Mike Rypins, just returned from Denmark as researcher for the Danish government; Jim Pappas, Modesto, Jersey breeder and editor of the *California Jersey News;* Don Brewer, herdsman at the University of Idaho; Morris Ketscher, owner of a 200-Holstein operation in Reedley, California; and Don Schuett, owner-manager of a cash-and-carry operation in Bakersfield, California.

Kansas State College Dairy Club Honors

F. W. ATKESON

(EDITOR'S NOTE: *F. W. Atkeson, 64, died April 4, 1958, at Riley County Hospital, Kansas. Another story appears in the "People and Events" Section.*)

The Second Annual Student-Faculty Banquet was held in the Student Union, March 11, 1958. Honored was professor and head of the dairy department, F. W. Atkeson. Due to illness, "Prof" was unable to attend in person. However, the entire banquet proceedings were put on tape and played back at the Atkeson home afterwards.

Toastmaster was Ray Schooley, dairy husbandry senior. Jack Van Horn, also a dairy husbandry senior, spoke for all the students and voiced their opinions. Jack mentioned that "Prof" was held in high regard by everyone in the department, whether undergraduate student or faculty. Dr. Glen Beck, director of the experiment station, was the speaker for the faculty. Musical entertainment was provided.

A committee composed of Ray Schooley, Lawrence Odgers, and Tom Townsend spent many hours preparing for the banquet. They contacted friends and former students in the state informing them of the event. They asked for replies in letter form, telling what these individuals could remember of their association with "Prof". Over one hundred answering letters were received and bound in book form for presentation to Professor Atkeson.

Also presented that night to "Prof" was a plaque which will hang in a place of honor on a wall of the herd book room. This is the inscription the plaque bears:

TO F. W. ATKESON

Distinguished educator, dairyman, judge, and builder of men,

Who has helped shape the lives of Kansas State Dairy students for the past quarter-century;

Who with wit and sincerity has taught countless students to realize and appreciate that they come to college for more than an education; and

Who with leadership and discipline has developed character, self-confidence, and common sense in students with whom he has been associated.

To influence so many lives and make so many friends is the privilege of few men—to do so with such dignity has made a place for him in the hearts of all his "boys".

In appreciation for all he has done, his students, past and present, offer this as a token of their sincere gratitude and deep admiration.

—*Kansas State College Dairy Club*

Every spring the Little American Royal is held. This is a student-managed fitting and showing contest that draws people from all over the state. Each year an outstanding man is chosen to be honored in the program book. This time, the dedication page honored F. W. Atkeson.

Winner of the Dairy Division was Lawrence Odgers, with a Guernsey cow. Reserve Champion went to Ray Schooley, who showed a

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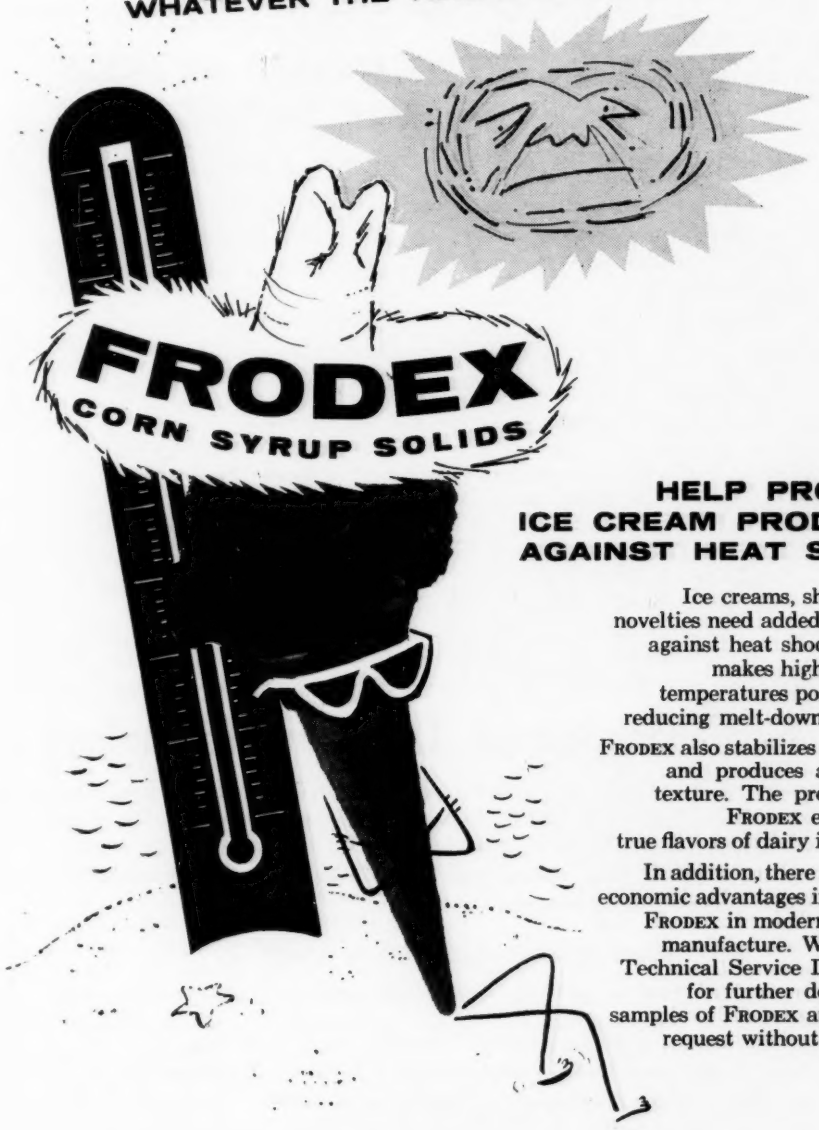

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Jersey cow. The show was held in the recently constructed Animal Industries Building, March 29. It was a success in its new surroundings.

Members not participating in the Little Royal served dinner to those attending the meeting at the Kansas Artificial Breeding Service Unit.

Where to Send It

Throughout the year many members and subscribers may have occasion to correspond with the Association about subscriptions to *The Journal of Dairy Science.*

So that all inquiries may be given prompt attention and quick action, the Association requests that you make a note of this brief outline of "where to send it."

Membership correspondence (including material relative to members' subscriptions to the Journal)—c/o H. F. JUDKINS, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, N.Y.

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SOME ARTICLES PUBLISHED IN 1956-57

"Chlorination of Wash Water in Relation to Butter Quality" by Dr. F. H. McDowall & A. K. R. McDowall

"Some Economic Problems of the Dairy Industry in Australia" by Dr. T. H. Strong

"Mechanization of Curd Fusion in Cheese Manufacture" by J. Czulak & L. A. Hammond

"Some Aspects of the Market Cream Industry in Australia" by E. G. Pont

"The Properties of Cryovac Packaged Cheddar Cheese" by T. A. Morris

"The Status of Milk Flavour Problems in the United States" by Dr. W. L. Dunkley

Obtainable through booksellers, or from the Australian Society of Dairy Technology, P. O. Box 20, Highett, Victoria, Australia. Price $3.50 per annum.

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Estimated by the U.S. Dept. of Agriculture: ARS-20-1, "Losses in Agriculture"

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Mention

Journal of Dairy Science

When Answering Advertisements

THIS HELPS BOTH THE ADVERTISER AND YOU

COMPOSITION AND NUTRITIVE VALUE OF CHEESE PRODUCED FROM MILK TREATED WITH HYDROGEN PEROXIDE AND CATALASE[1]

L. J. TEPLY, P. H. DERSE, AND W. V. PRICE

The Wisconsin Alumni Research Foundation and the Department of Dairy Industry, University of Wisconsin, Madison

SUMMARY

This study provides information on the nutritional value and wholesomeness of cheese made from milk treated with hydrogen peroxide at 5, 10, and 25 times the anticipated normal practical levels. The milks and their wheys obtained in preparing the cheese samples also were tested. The samples were analyzed for proximate composition and for the vitamins, niacin, thiamine, riboflavin, vitamin B_6, pantothenic acid, folic acid, vitamin B_{12}, vitamin A, and beta-carotene. The amino acids, methionine, cystine, tryptophane, and lysine, also were determined. Biological protein evaluation was done for milk, whey, and cheese by feeding these products to rats to provide 9% protein (dry-weight basis) as the sole source of protein in an otherwise complete ration. Additional rat-feeding tests were done on cheese which furnished 14% protein in the diet. The results indicated no marked changes in the composition or nutritive value of milk treated with 0.1, 0.2, and 0.5% hydrogen peroxide, or of the whey or cheese obtained from that milk.

Various dairy products have been treated with hydrogen peroxide for many years and the literature on this subject has been reviewed recently *(7)*. It has been reported that treatment of milk with low levels of hydrogen peroxide for short periods of time (with subsequent enzymatic removal of residual hydrogen peroxide) enables production of cheese of superior quality *(1, 9)*. The beneficial effects of the peroxide appear to be owing to a selective reduction in the population of certain bacteria.

This study was initiated to provide information on the effect of treatment of milk with hydrogen peroxide on the composition and nutritive value of cheese made from that milk. Analysis for proximate composition, vitamins, and amino acids, and biological protein evaluation (rat-feeding) tests were carried out. The cheese samples were made from raw, pasteurized, and 120° F.–treated milks (no peroxide treatment), and from pasteurized and 120° F. milks processed with 5, 10, and 25 times the anticipated normal practice levels of hydrogen peroxide and for approximately 25 times the anticipated treatment period, considering the temperature employed during treatment. The milks themselves, and the wheys obtained from the making of the cheese samples, also were subjected to analysis and biological protein evaluation. Electrophoretic studies were carried out on proteins obtained from the cheese samples.

Received for publication August 13, 1957.

[1] This work was supported by a financial grant from the National Cheese Institute, Chicago, Illinois. The N.C.I. also made arrangements for carrying out the electrophoretic studies.

MATERIALS AND METHODS

Preparation of test materials. The facilities of the University of Wisconsin at Babcock Hall were used for the cheese-making and preparation and packaging of samples. All analyses were carried out promptly on samples held under refrigeration. Samples for rat-feeding tests were stored in a freezer.

The milk used in these experiments was taken from the mixed daily supply from 25 herds. The milk received on October 22, 1956, was clarified, cooled, and treated the next morning.

Cheese was made from raw milk, from milk heated to 120° F., and from pasteurized milk. Portions of the 120° F. and pasteurized milks were further subjected to hydrogen-peroxide-catalase treatments. In all cases, the peroxide treatment was carried out at 120° F. The various portions of milk, each of which weighed 480 lb., were handled as follows: One portion of the mixed raw milk was placed in a 60-gal. cheese vat for manufacturing. Approximately 2,000 lb. of milk was heated to 120° F. in a plate-type heater and run into a 300-gal. tank. One portion of this hot milk was immediately cooled from 120 to 88° F., for cheese-making.

The remainder of the 120°–heated milk in the tank was divided into three 480-lb. portions. To one was added 0.1% hydrogen peroxide, to the second 0.2%, and to the third portion, 0.5%. These percentages represent 100% H_2O_2. (For actual amounts of 35% H_2O_2 used to provide these levels of peroxide, see Table 1.) After holding 10 min. under continuous agitation, each portion

TABLE 1

Amounts of hydrogen peroxide and catalase used in 480-lb. vat-lots of milk

Level of peroxide	H_2O_2 required	35% Peroxide used [a]	Catalase used [b]
(%)	*(lb.)*	*(lb.)*	*(ml.)*
0.5	2.40	6.85	44
0.2	0.96	2.74	17
0.1	0.48	1.37	9

[a] The required amount of 35% hydrogen peroxide was mixed with an equal weight of cold water before mixing with the milk.

[b] The required amount of catalase preparation was mixed with five times its volume of cold water before mixing with the milk.

was cooled from 120 to approximately 90° F. and the catalase preparation was added, to destroy the remaining hydrogen peroxide. Each lot of milk was then adjusted to 88° F. for cheese-making.

A second 2,000-lb. portion of the mixed milk was heated in a high temperature, short-time (HTST) pasteurizer to 162° F. for 20 sec., cooled to 110° F., and delivered into a 300-gal. tank, from which one portion was drawn and cooled promptly to 88° F. for cheese-making.

The pasteurized milk remaining in the tank was warmed to 120° F. and divided into three 480-lb. portions. The peroxide treatment of these portions was carried out as just described for the 120° F. milk.

After the pasteurized milk had been divided for the peroxide treatments, an

error in handling made it necessary to discard one of the 480-lb. portions. An equal amount of milk pasteurized by the same method had to be substituted for it. This substitute lot contained a small portion of the milk delivered to the manufacturing laboratory on the day of the experiment. This substitute portion of pasteurized milk was the one treated with 0.2% of hydrogen peroxide.

The hydrogen peroxide used in these experiments was a 35% solution of H_2O_2 designed for use in food processing. The peroxide solution was diluted with an equal weight of cold water before it was added to the milk. The catalase preparation used in these experiments was a stable, buffered solution, at neutral pH, which had been standardized to a potency of 100 Keil Units per cubic centimeter. One Keil unit of catalase is defined as the quantity of catalase required to decompose 1 g. of 100% hydrogen peroxide in 10 min. at 25° C. in an inert atmosphere of CO_2 or N_2. Four times the required theoretical amount of catalase was used to remove all traces of hydrogen peroxide from the milk within 10 min. The catalase preparation was diluted with five times its volume of cold water, then added slowly to the milk, with constant agitation to prevent excessive foaming. The specific amounts used for each concentration of hydrogen peroxide are shown (Table 1). All the peroxide in the treated milk was removed by the catalase before starting cheese-making. The milk was tested for the presence of hydrogen peroxide by adding several drops of a fresh 25% solution of potassium iodide with 2% starch to 5 ml. of milk in a tube. A control tube of identical milk not treated with the hydrogen peroxide was prepared similarly. Complete destruction of hydrogen peroxide was indicated by identical freedom from discoloration in both tubes.

The whey for analysis was removed from each experimental vat just before draining; it was promptly cooled and held at 36° F. To provide whey protein for biological evaluation the following procedure was used:

Unseparated whey removed from the cheese vats at the time of dipping was heated to 200° F. The whey proteins were precipitated by adding to each 100 lb. of the hot whey, 33 ml. of glacial acid diluted with water to 100 ml. The whey-protein precipitate was drained at 70° F. on "Viskon," thoroughly mixed for uniformity, packed in heavily paraffined cottage cheese cartons, fast-frozen at −25° F., and stored at from −10 to −20° F.

All lots of cheese were formed into 5-lb. loaves, wrapped in "Parakote," and held in fiberboard cartons.

The time schedule and method of making the milk into cheese followed procedures which have been described elsewhere *(16)* (Table 2). The time intervals planned for these making procedures required a pH of 6.1 in the curd, at 2.5 hr. after adding rennet to the milk, and a pH of 5.5 at milling, at 4.5 hr. after renneting. When time and acidity measurements did not fit the ideal schedule exactly, then the acidity attained determined the time of dipping or milling. Measurements of pH of curd were made with a Leeds-Northrup portable potentiometer, quinhydrone electrode, and saturated calomel half-cell *(16)*.

Preliminary experiments had shown the desirability of increasing the amount of rennet used in making the cheese from milk treated with these high levels

TABLE 2

Times required for critical stages in the making operation

Milk treatment	Setting[a] to dipping[b]	Dipping to milling[c]	Setting to milling
	hr:min.	hr:min.	hr:min.
Raw milk	2:50	2:30	5:20
Pasteurized			
0.5% H_2O_2	3:08	2:25	5:33
0.2% H_2O_2[d]	3:00	2:16	5:16
0.1% H_2O_2	2:45	2:03	4:48
0% H_2O_2	2:46	1:40	4:26
Heated to 120° F.			
0.5% H_2O_2	3:35	2:10	5:45
0.2% H_2O_2	3:25	2:00	5:25
0.1% H_2O_2	3:21	2:25	5:46
0% H_2O_2	2:45	2:38	5:23

[a] Setting (adding of rennet) occurred when the Marschall rennet test showed ¼-space-change in 15 min.

[b] Dipping (end of running whey) was at pH 6.1.

[c] Milling was at pH 5.5.

[d] This vat-lot of milk was not identical to the others in this series.

of hydrogen peroxide. Therefore, 4 oz. of rennet per 1,000 lb. of milk was used in all lots of milk treated with hydrogen peroxide; 3 oz. per 1,000 lb. was used in the other lots. Under these conditions, milk coagulation was normal.

Development of acidity was slightly slower at first, in vats to which the peroxide had been added. This was especially noticeable in those lots made with 120°–heated milk. This effect might have been prevented by prolonging the period between adding starter and the setting of the vat.

Curd observed at all stages of making was slightly lacking in firmness in those lots which had received 0.5% of hydrogen peroxide. This tendency was not detected in other vat-lots.

For the later feeding of cheese to rats, at a level sufficient to furnish 14% protein in the diet, additional cheese samples were made from pasteurized milk according to the general procedures described above, except that only one level of hydrogen peroxide (0.5%) was used, and the treatment was at 120° for 10 and 30 min. All of the milks were held for an equal period at 120° F. Duplicate sets of samples were made on two separate days and from two different batches of milk.

Analytical methods. The analytical methods employed were, for the most part, well-standardized procedures: moisture *(2)*, ash *(2)*, fat *(2)*, protein *(2)*, carbohydrate (by difference), niacin *(10)*, thiamine *(16)*, riboflavin *(11)*, vitamin B_6 *(1)*, pantothenic acid *(14)*, folic acid *(2)*, vitamin B_{12} *(11)*, vitamin A and beta-carotene *(6)*, methionine, cystine, tryptophane, and lysine *(5)*. For the cheese samples, special methods were used for determining vitamin A and beta-carotene *(2, 6)*, moisture *(13)*, and fat *(12)*. The latter was determined by a modified Babcock test procedure. Hartman *et al. (4)* have demonstrated that cheese requires special treatment for vitamin B_{12} assay; the cheese samples were extracted with acetone and the extracts were then carried through the regular assay procedure.

Biological protein evaluation. Weanling (21-day-old) male albino rats of the Sprague-Dawley strain, weighing 50-60 g., were divided into groups of ten rats each, in such a way that the average weights of all the groups were essentially the same. The animals were housed in individual screen-bottom cages in air-conditioned, temperature-controlled animal quarters; they were supplied with water and test ration ad libitum.

Each sample was incorporated into a semisynthetic rat basal ration as the sole protein source, at such a level as to supply 9.09% protein ($N \times 6.25$) in the finished ration (dry basis). The rations were equalized with respect to fat, ash, carbohydrate, and moisture content, and supplemented with a synthetic vitamin mixture. All of the whey proteins tested contained approximately the following percentages: protein 13, moisture 75, fat 7, ash 0.7, and carbohydrate 4. The casein standard contained 91.3% protein. The final rations for the milk groups contained per 100 g. of solids, 260 g. of moisture; for the whey groups, 60 g., and for the cheese groups, 20 g. In all other respects the rations were identical. The cheese, whey protein, and milk samples were stored frozen. Rations were made up weekly and held in a refrigerator.

The test animals were weighed at weekly intervals through the 6 wk. test period and were fed weighed amounts of test ration. Uneaten rations removed from the cages were collected, combined and, at intervals analyzed for protein and solids content, so as to obtain an accurate estimation of the total food consumption and protein consumption of the animals.

Diet

	(%)
Protein (from sample)	9.09
Corn oil (plus sample fat)	12
Salts I (USP IV) (plus sample ash)	4
Vitamin-glucose mixture[a]	1
Sucrose to	100

[a] Each 100-g. ration provided U.S.P. units: vitamin D, 200, vitamin E, 101, vitamin A, 2,000. Milligrams: choline, 100, inositol, 10, niacin, 4, calcium pantothenate, 4, vitamin B_{12}, 0.003, riboflavin, 0.8, thiamine, 0.5, vitamin B_6, 0.5, menadione, 0.5, folic acid, 0.2, and biotin, 0.04; *para*-aminobenzoic acid, 10 grams.

Electrophoretic studies. The electrophoretic studies were carried out at the National Dairy Research Laboratories, Inc., Oakdale, L. I., New York.

Two grams of cheese were weighed out and triturated with ether until the major portion of the fat was removed. The cheese samples were put into solution in sodium cacodylate–sodium chloride buffer (NaCaCO, 02M; NaCl, 0.08M), 0.1 μ, pH 6.63. An excess of disodium dihydrogen versenate was added and the pH was adjusted to 7.3 with 0.1 *N* NaOH for maximum sequestering of the calcium present. All the protein went into solution, but residual fat caused turbidity. The solutions were ether-extracted at room temperature until sufficiently clear for analysis. The samples were dialyzed against three changes

of buffer for three days at a temperature of 4° C. Analyses were made at a temperature of 1° C. in a Tiselius-type electrophoresis apparatus manufactured by Frank Pearson Associates. Areas of the components were measured by planimeter, and mobilities were calculated from the descending boundaries. Measurements for mobility were made from the intial boundary to the ordinate bisecting the area of a component.

Feeding tests at a 14% protein level. Feeding tests were carried out with newly prepared samples of cheese which had been treated for 10 and 30 min. with 0.5% H_2O_2. The tests were carried out in the same way as the protein evaluation tests, except for the higher level of sample, to furnish a 14% level of protein; the total fat level was 18%.

RESULTS AND DISCUSSION

Analysis. The analytical results on the milk, cheese, and whey samples are presented (Tables 3, 4, and 5). For the most part, the values fall into the ranges one might expect from data available in the literature. However, in the case of certain nutrients, such as folic acid, which are present at rather low levels in relation to the sensitivity of the test methods, the variation in values among the different milk, cheese, and whey samples is somewhat greater than one would expect from normal assay variation. The microbiological assay procedures employed for the amino acid determinations and most of the vitamin assays are ordinarily considered to give results reproducible, on the average, within a range of ± 10%. For some vitamins, such as folic acid, one expects somewhat greater variation in assay results.

In general, there is no effect of peroxide treatment on levels of nutrients. However, in the case of methionine and lysine, treatment of milk with 0.5% H_2O_2 resulted in a decrease of the levels of these amino acids in cheese produced from this milk, to the extent of 10–25% (dry weight basis). This effect appears to be an indirect one, as it was not noted in the milk and whey samples.

These results substantiate other data which have suggested that, aside from ascorbic acid, the nutrients in milk are rather insensitive to treatment with hydrogen peroxide. Vitamin A in milk has been reported to be relatively stable to peroxide treatment *(14)* and Nambudripad *et al. (10)* found thiamine, riboflavin, niacin, and vitamin B_{12} to be unaffected by addition of 0.03% H_2O_2 (by weight) and by allowing the milk to stand for from 1 to 2 hr. before pasteurizing at 145° F.

Although strong treatment with hydrogen peroxide will produce chemical changes in proteins and amino acids of milk, relatively mild treatment is reported to have little effect *(8, 17)*. This is borne out by the results of the amino acid assays and the electrophoretic studies reported herein.

Protein evaluation. The rats on the protein test were in good health during the experiment and no abnormalities were found on autopsy at termination. Good protein efficiency values (Table 6) were obtained on the various samples and the variation among samples was not unusual for biological tests, with one exception: the protein efficiency ratio for the heat-treated 0.5% peroxide cheese

TABLE 3

TABLE 3

Analysis of milk

	Raw	Pasteurized (160° F., 20 sec.) (% H_2O_2)			120° F.–treated (% H_2O_2)			
		0	0.1	0.2	0.5	0	0.1	0.2	0.5
Moisture *(%)*	88.55	88.17	88.65	88.64	88.42	88.70	87.88	88.39	89.79
Ash *(%)*	0.74	0.74	0.74	0.72	0.69	0.73	0.72	0.73	0.72
Fat *(%)*	3.30	3.43	3.32	3.47	3.41	3.26	3.49	3.46	3.10
Protein *(%)*	3.25	3.38	3.32	3.32	3.32	3.19	3.51	3.25	3.25
Carbohydrate *(%)*	4.16	4.28	3.97	3.85	4.16	4.12	4.40	4.17	3.14
Niacin *(mg/100 ml.)*	0.084	0.100	.....	.....	0.084	0.078	.....	.....	0.096
Thiamine *(mg/100 ml.)*	0.044	0.047	.....	.....	0.039	0.036	.....	.....	0.038
Riboflavin *(mg/100 ml.)*	0.16	0.17	.....	.....	0.16	0.17	.....	.....	0.18
Vitamin B_6 *(mg/100 ml.)*	0.075	0.064	0.074	0.076	0.073	0.082	0.074	0.055	0.051
Pantothenic Acid *(mg/100 ml.)*	0.45	0.44	.....	.....	0.45	0.43	.....	.....	0.45
Folic Acid *(μg/100 ml.)*	0.57	0.41	.....	.....	0.46	1.14	.....	.....	0.54
Vitamin B_{12} *(μg/100 ml.)*	0.38	0.34	0.46	0.41	0.43	0.46	0.44	0.37	0.32
Vitamin A *(I.U/100 ml.)*	98.3	91.7	.....	.....	93.3	88.3	.....	.....	95.0
Beta-carotene *(μg/100 ml.)*	25.5	26.0	.....	.....	24.7	26.0	.....	.....	24.5
Methionine *(mg/ml)*	0.75	0.75	.....	.....	0.68	0.78	.....	.....	0.70
Cystine *(mg/ml)*	0.21	0.20	.....	.....	0.19	0.22	.....	.....	0.22
Tryptophane *(mg/ml)*	0.44	0.43	.....	.....	0.40	0.40	.....	.....	0.38
Lysine *(mg/ml)*	2.9	2.9	.....	.....	2.8	2.8	.....	.....	3.0

TABLE 4

Analysis of cheese

	Raw	Pasteurized (160° F., 20 sec.) (% H_2O_2)			120° F.–treated (% H_2O_2)			
		0	0.1	0.2	0.5	0	0.1	0.2	0.5
Moisture *(%)*	38.07	37.69	40.66	40.90	41.24	38.22	39.96	39.75	41.55
Ash *(%)*	4.05	3.65	3.41	3.60	3.68	3.26	3.77	3.56	3.69
Fat *(%)*	32.6	32.4	31.0	30.2	31.0	32.3	31.4	30.8	30.3
Protein *(%)*	27.24	26.09	25.07	26.09	25.33	26.54	24.31	25.46	26.16
Niacin *(mg/100 g.)*	0.080	0.086	.....	.....	0.084	0.092	.....	.....	0.070
Thiamin *(mg/100 g.)*	0.097	0.030	.....	.....	0.020	0.023	.....	.....	0.024
Riboflavin *(mg/100 g.)*	0.48	0.43	.....	.....	0.32	0.38	.....	.....	0.38
Vitamin B_6 *(mg/100 g.)*	0.078	0.077	0.086	0.080	0.091	0.098	0.108	0.102	0.109
Pantothenic Acid *(mg/100 g.)*	0.49	0.51	.....	.....	0.52	0.41	.....	.....	0.43
Folic Acid *(mg./100 g.)*	0.038	0.037	.....	.....	0.044	0.033	.....	.....	0.059
Vitamin B_{12} *(μg/100 g.)*	0.54	0.78	0.74	0.62	0.95	1.33	1.54	0.61	0.58
Vitamin A *(I.U/100 g.)*	1,000	1,050	.....	.....	900	1,000	.....	.....	900
Beta-carotene *(μg/100 g.)*	200	220	.....	.....	190	195	.....	.....	175
Methionine *(mg/g)*	7.07	7.40	.....	.....	5.42	6.73	.....	.....	5.22
Cystine *(mg/g)*	1.00	1.00	.....	.....	0.85	1.02	.....	.....	0.88
Tryptophane *(mg/g)*	2.9	5.0	.....	.....	3.0	3.1	.....	.....	3.0
Lysine *(mg/g)*	25.2	25.0	.....	.....	20.9	23.8	.....	.....	22.0

TABLE 5

Analysis of whey

	Raw	Pasteurized (160° F., 20 sec.) ($\%$ H_2O_2)				120° F.–treated ($\%$ H_2O_2)			
		0	0.1	0.2	0.5	0	0.1	0.2	0.5
Moisture *(%)*	93.40	93.42	93.46	93.36	93.65	93.46	93.54	93.56	93.45
Ash *(%)*	0.56	0.56	0.58	0.57	0.54	0.54	0.57	0.58	0.56
Fat *(%)*	0.34	0.27	0.26	0.32	0.33	0.33	0.33	0.40	0.37
Protein *(%)*	1.02	0.96	0.96	1.02	1.08	1.02	1.02	1.08	1.08
Carbohydrate *(%)*	4.68	4.79	4.74	4.73	4.40	4.65	4.54	4.38	4.54
Niacin *(mg/100 ml.)*	0.060	0.074	.....	.....	0.120	0.074	.....	.....	0.098
Thiamine *(mg/100 ml.)*	0.049	0.066	.....	.....	0.056	0.043	.....	.....	0.043
Riboflavin *(mg/100 ml.)*	0.13	0.12	.....	.....	0.14	0.13	.....	.....	0.13
Vitamin B_6 *(mg/100 ml.)*	0.059	0.058	0.062	0.031	0.061	0.063	0.058	0.061	0.057
Pantothenic Acid *(mg/100 ml.)*	0.35	0.39	.....	.....	0.37	0.39	.....	.....	0.32
Folic Acid *(μg/100 ml.)*	5.2	2.4	.....	.....	4.4	5.0	.....	.....	4.3
Vitamin B_{12} *(μg/100 ml.)*	0.24	0.24	0.28	0.20	0.24	0.25	0.30	0.18	0.21
Methionine *(mg/ml)*	0.17	0.17	.....	.....	0.17	0.17	.....	.....	0.17
Cystine *(mg/ml)*	0.18	0.19	.....	.....	0.15	0.17	.....	.....	0.18
Tryptophane *(mg/ml)*	0.14	0.15	.....	.....	0.14	0.15	.....	.....	0.13
Lysine *(mg/ml)*	0.83	0.83	.....	.....	0.83	0.82	.....	.....	0.89

sample was 1.82, compared to a value of 2.34 for the control. In a repeat test on this sample (which had been stored in a freezer), a protein efficiency ratio of 2.14 was obtained, with a value of 2.40 for the control. The results as a whole indicate little, if any, change in the nutritional value of cheese protein when the milk from which it is made is treated with peroxide as described.

Electrophoresis. The electrophoretic analyses of proteins of cheeses made from milk treated with various levels of H_2O_2 are shown (Table 7 and Figure 1). The cheese samples were stored in the frozen state for approximately 2 mo., then stored at 40° F. for up to 2 wk. before examining. The four samples which had the longest storage periods *(1, 2)* were all low in concentration of beta-casein in the descending patterns, indicating possible storage changes. The descending patterns of the pasteurized series have marked similarity in appearance. Samples 2 and 4, treated, respectively, with the lowest and highest concentration of H_2O_2, show no real differences in their analyses. The control Sample 1 and Sample 3 (0.2% H_2O_2) are both low in beta-casein in the descending pattern. This may be due to the slightly longer storage periods for these samples. The mobility of the alpha-casein complex in the control Sample 1 seems rather low compared to the other pasteurized samples. This difference can not be considered as the result of the peroxide treatment specifically, since Samples 5 (120° F., no H_2O_2) has mobilities similar to the peroxide-treated samples.

In the 120° F. series, no definite changes due to the peroxide treatment were noticed. As in the pasteurized series, the samples with the longest storage periods were low in concentration of beta-casein in the descending patterns. All samples, both pasteurized and 120° F.–treated, showed some dissociation of the alpha-casein complex in the ascending pattern. The degree of dissociation was not related to the strength of the peroxide treatment.

The absence of an effect on electrophoretic patterns, definitely attributable to the peroxide treatments used in these experiments, is what we might expect from electrophoretic studies on peroxide-treated milk reported by Lück and Joubert *(8)*. These workers did observe some changes in the β-lactoglobulin diagrams when skimmilk was treated with 0.25 and 1% H_2O_2 (39%) for from 3 to 5 hr. at room temperature, at which time the catalase was inactivated. After the catalase inactivation, the H_2O_2 level remained constant at from 0.015 to 0.10 *M*. The milk was held for from four to six days after catalase inactivation. Lück and Joubert note that other workers have observed similar effects on the β-lactoglobulin from other treatments, such as radiation with ultraviolet light, or heat treatment.

Feeding tests at a 14% protein level. All rats were in good health during the experiment and no abnormalities were found on autopsy at termination. Analyses of the cheese are given (Table 8) and weight gain and food consumption data also are presented (Table 9). On the average, the animals on the peroxide-treated cheeses gained from 5 to 10% less than the animals on the control cheeses. Statistical analysis showed that variation in feed efficiency among groups was not significantly different (at the 5% level) from variation in feed efficiencies within the groups. There was good correlation between weight gain

TABLE 6

Biological protein evaluation results

(Rat weights are averages for ten rats—in grams)

Milk treatment (% H_2O_2):	Raw	Pasteurized (162° F., 20 sec.)				120° F–treated			
	0.0	0.0	0.1	0.2	0.5	0.0	0.1	0.2	0.5
Milk samples									
Initial weight	53.3	53.6	53.7	52.5	53.2	53.4	52.5	52.3	52.0
6-wk. weight	176.9	185.6	175.6	170.7	173.8	192.9	178.9	185.4	179.5
Food consumption[a]	457.7	132.0	121.9	118.2	120.6	139.5	126.4	133.1	127.5
Protein efficiency[b]	3.04	498.1	479.1	461.2	483.2	514.5	498.0	517.2	489.7
Total gain	123.6	3.00	2.88	2.95	2.94	3.01	2.82	2.97	2.94
Whey protein samples									
Initial weight	52.5	52.7	52.5	52.6	52.7	52.8	52.4	52.3	52.7
6-wk. weight	200.4	206.3	187.0	194.6	183.9	188.0	209.3	189.4	202.2
Total gain	147.9	153.6	134.5	142.0	131.2	135.2	156.9	137.1	149.5
Food consumption[a]	505.9	525.1	474.7	496.8	485.7	490.8	503.3	474.0	488.1
Protein efficiency[b]	3.19	3.19	3.04	3.12	2.92	2.97	3.43	3.16	3.36
Cheese samples									
Initial weight	51.9	52.0	51.3	51.7	52.1	52.5	51.1	52.9	52.9
6-wk. weight	129.6	140.1	140.6	124.4	123.6	136.8	137.4	135.9	111.3
Total gain	77.7	88.1	89.3	72.7	71.5	84.3	86.3	83.0	58.4
Food consumption[a]	361.9	377.5	402.0	352.8	359.7	380.9	378.3	377.6	318.0
Protein efficiency[b]	2.27	2.52	2.42	2.20	2.10	2.34	2.49	2.33	1.82

Casein standards:	A[c]	B[d]		Repeat test on cheese from heat-treated milk: H_2O_2	Repeat test on cheese from heat-treated milk: 0.5% H_2O_2
Initial weight	52.4	51.8	Initial weight	55.0	54.8
6-wk. weight	145.8	172.3	6-wk. weight	136.8	120.8
Total gain	93.4	120.5	Total gain	81.8	65.2
Food consumption	404.1	443.5	Food consumption	353	323
Protein efficiency	2.33	2.82	Protein efficiency	2.40	2.14

[a] Dry basis.
[b] Grams gain per gram protein consumed.
[c] A = diet moisture content as for whey diet.
[d] B = diet moisture content as for cheese diet.

TABLE 7

Electrophoretic analyses of Cheddar cheese proteins

Sample	Date of analysis	Treatment	Rel. concentration ascending	Rel. concentration descending	Mobility descending
			(%)		(1×10^5 cm²/sec volt)
1	1/24/57	Pasteurized, no H_2O_2	A[a] 31.4 B[a] 62.2 C[a] 7.3	A 17.3 B 82.7	A −1.90 B −4.50
2	1/15/57	Pasteurized, 0.1% H_2O_2	A 37.0 B 49.7 C 13.3	A 28.2 B 71.8	A −2.28 B −5.23
3	2/1/57	Pasteurized, 0.2% H_2O_2	A 24.3 B 67.3 C 8.4	A 18.1 B 63.8 C 18.1	A −1.70 B −4.41 C −6.78
4	1/17/57	Pasteurized, 0.5% H_2O_2	A 33.0 B 51.8 C 15.2	A 30.0 B 70.0	A −2.08 B −5.06
5	1/14/57	120° F., no H_2O_2	A 32.9 B 53.9 C 13.2	A 27.0 B 73.0	A −2.32 B −5.28
6	1/22/57	120° F., 0.1% H_2O_2	A 29.8 B 52.2 C 18.0	A 18.0 B 82.0	A −2.25 B −5.19
7	1/21/57	120° F., 0.2% H_2O_2	A 35.3 B 55.4 C 9.3	A 22.4 B 63.4 C 14.2	A −2.20 B −4.76 C −6.22
8	1/18/57	120° F., 0.5% H_2O_2	A 33.5 B 49.1 C 17.4	A 30.1 B 69.9	A −2.10 B −5.06

Buffer: Na cacodylate (0.02 *M*) NaCl(0.08*M*), pH 6.63, 0.1 U.
Av. E = 4.90 volts/cm.
Av. T = 15,547 sec.
[a] Components: A = beta-casein, B = alpha-casein complex, C = dissociated fraction from alpha-casein complex.

TABLE 8

Analysis of cheese used in feeding tests at a 14% protein level.

	Protein	Fat	Moisture	Ash
		(%)		
7-16 Control	25.9	31.1	38.4	4.24
7-17 Control	25.1	31.7	37.5	4.86
7-16 0.5% H_2O_2—10 min.	23.5	28.9	42.9	3.95
7-17 0.5% H_2O_2—10 min.	22.8	28.8	42.9	3.64
7-16 0.5% H_2O_2—30 min.	23.0	28.6	43.2	4.01
7-17 0.5% H_2O_2—30 min.	22.0	28.0	43.2	4.36

TABLE 9

Feeding trials with cheese furnishing 14% protein

	Average initial weight	Average weight after 6 wk.	Average weekly weight gain	Average weekly feed consumption
		(g.)		
Control, 7-16	63.2	259.9	32.8	116.8
Control, 7-17	63.1	256.7	32.3	119.3
H_2O_2—10 min., 7-16	63.1	247.8	30.8	116.0
H_2O_2—10 min., 7-17	63.2	241.1	29.6	110.9
H_2O_2—30 min., 7-16	63.1	239.3	29.4	113.3
H_2O_2—30 min., 7-17	63.1	246.2	30.5	119.3

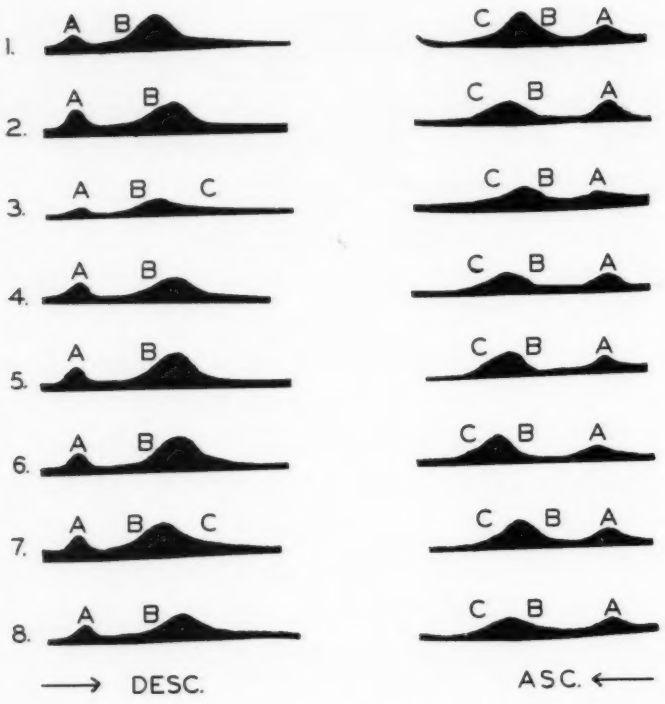

FIG. 1. Electrophoresis of proteins of cheeses made from milks treated with various levels of hydrogen peroxide. The sample treatments are given (Table 7).

and feed consumption, both among groups and within each group. The moderate difference in growth between control and experimental groups was apparently related to a slight difference in feed consumption.

CONCLUSIONS

Analytical values for milk, cheese, and whey samples were in good agreement with published values. In general, there was no effect of peroxide-catalase treatment of milk on levels of nutrients in the milk, or in cheese or whey obtained from that milk. Considering the data on a dry-weight basis, treatment of milk with 0.5% peroxide lowered the values for cystine and methionine in the cheese by from 10 to 25%.

Good protein efficiency ratios were obtained on all of the milk, cheese, and whey samples, and variation was within normal limits with one exception, in the case of the cheese made from 120° F. 0.5% peroxide-treated milk. However, the definite lowering of the protein efficiency ratio indicated in the first test on this sample was not confirmed in a repeat test on this particular sample.

Electrophoretic studies on proteins from the cheese samples showed only

minor differences, which did not appear to be directly related to the peroxide treatment.

The rat-feeding tests carried out on duplicate sets of cheese made from milk treated with 0.5% hydrogen peroxide for 10 and 30 min., and with the cheese furnishing 14% protein diet, showed only a slight difference in growth between control and experimental groups.

The results as a whole indicate no marked changes in the composition or nutritional value of milk treated with 0.1, 0.2, or 0.5% hydrogen peroxide under the conditions described, or in the cheese or whey obtained from that milk.

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DISTRIBUTION OF PROPIONIBACTERIA IN SWISS CHEESE

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SUMMARY

Conventional wheel and rectangular rind-bearing block Swiss cheeses were studied to determine the distribution of propionibacteria. Samples were taken from three predetermined locations in six different Swiss cheeses which were from 1 to 16 mo. old. Propionibacteria were determined by the plating method of Kambar *et al. (3)*, and cultures were taken from agar plates and identified. Few propionibacteria were found in samples taken 1/8-in. below the surface of the cheese. With one exception, samples taken 7/8-in. below the surface contained many more propionibacteria than were found in the exterior portions. Proceeding inward to sections 2 5/8 in. below the cheese surface, the numbers increased, though not proportionally. These data suggest that the decrease in numbers of propionibacteria toward the exterior of Swiss cheese is probably the result of such unfavorable conditions as lower moisture, higher salt concentrations, and higher oxidation-reduction potentials.

From a bacteriological aspect, the outstanding characteristic of Swiss cheese is the presence of large numbers of propionic acid bacteria. The role of these organisms in the development of flavor in Swiss cheese has been clearly established by many workers. In numerous instances, the numbers of these organisms in cheese at various stages of the make and curing operation have been determined. No studies have been reported which have shown the distribution of propionibacteria within individual samples of Swiss cheese. The object of this investigation was to determine the influence of the usual make procedures upon the distribution of propionibacteria in Swiss cheese.

EXPERIMENTAL PROCEDURE

Cheeses. Information pertaining to the identification of the cheeses used in this study is shown (Table 1). Cheeses W1 through W4 were conventional wheel-shaped Swiss cheeses obtained through a commercial source. Cheeses B11 and B12 were experimental cheeses which differed from conventional Swiss cheese only in their shape; these were rectangular blocks, each weighing approximately 80 lb. Flavor and eye development in each cheese was considered good by a competent judge.

Sectioning cheese for analysis. A 4-in.-wide cross section was cut vertically through the middle of the cheese; from this section three layers parallel to the top surface were removed for analysis. The first section consisted of a layer 1/4-in. deep taken 1/8-in. under the surface of the cheese. This section hereafter is referred to as Position 1. Then, 1/2-in. of cheese was removed and discarded. A layer 1/4-in. deep was then taken; this section is referred to as Position 2. The next 1 1/2 in. of cheese was removed and discarded. The next layer, 1/4-in.

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TABLE 1
Identification and description of experimental Swiss cheeses

Cheese No.	Form	Age
		(mo.)
B11	80-lb. Block	1
B12	80-lb. Block	1
W1	Wheel	2
W2	Wheel	2
W3	Wheel	16
W4	Wheel	16

deep, is referred to as Position 3. An 11-g. sample for plating purposes was taken from that portion of each layer that approximated the center of the cheese as viewed from the surface. Adjacent portions were taken for chemical analysis.

Bacteriological analysis. The cheese used for plating was weighed (11 g.) into a sterile container, then ground in a mortar to a smooth mass. During the grinding, 99 ml. of sterile aqueous 2.0% sodium citrate was added. Grinding was continued until a milky homogenous suspension resulted.

The plating method of Kambar *et al. (3)* was used. Duplicate plates were prepared. After counting, one plate which represented the proper dilution and possessed uniformly distributed bacterial colonies was selected for each cheese sample.

One hundred adjacent colonies from each plate were inoculated into tubes of the broth described by Kambar *et al. (3)* and were incubated at 30° C. After sufficient growth had occurred, the cultures were examined morphologically, using the gram stain. Cultures were tested for catalase production by combining 1 ml. of broth culture with 1 ml. of 3.0% hydrogen peroxide in a clean test tube. Cultures showing any evolution of gas were considered catalase positive. The ability of the cultures to ferment sodium lactate was determined. The medium used in these studies contained 0.5% Difco yeast extract, 2.0% B.B.L. Trypticase, 1.0% sodium lactate, and 1 ml. of a 1.6% solution of brom thymol blue. These ingredients were dissolved in distilled water; the broth was then adjusted to pH 7.0 with sodium hydroxide. Sterilization was accomplished by heating at 121° C. for 20 min. Cultures developing acidity in this medium were considered capable of fermenting sodium lactate. The appearance of the growth in broth developed by each colony was also recorded, as an aid toward classification.

Total aerobic counts were made, using the carrot-liver extract medium of Barber and Frazier *(2)*. This medium was modified slightly. B.B.L. Trypticase was used in place of Difco Neopeptone and the agar content was increased from 1.2 to 1.5%.

Chemical analysis. Standard methods *(1)* for the determination of moisture and salt were used. Samples for the determination of moisture were not preheated on a steam bath. Instead, samples were preheated in an oven without vacuum for 15 min. at 100° C.

RESULTS

The distribution of propionibacteria in Swiss cheese followed a definite pattern (Table 2). In all of the six cheeses examined, the propionibacteria de-

TABLE 2

Bacterial content and composition of six Swiss cheeses

Designation of cheese and sample location	Depth of sample from surface	Propioni-bacteria[a]	Total aerobic count[a]	Moisture content of cheese sample	Salt content of cheese sample	Salt in moisture
	(in.)	*(per g.)*		*(%)*		
B11–1	⅛	1	35	24.72	2.03	8.21
B11–2	⅞	700	186	36.60	1.27	3.47
B11–3	2⅝	1,350	246	38.77	0.31	0.80
B12–1	⅛	1	35	25.54	2.35	9.20
B12–2	⅞	1,200	151	35.74	1.29	3.61
B12–3	2⅝	2,170	189	38.19	0.23	0.60
W1–1	⅛	1	98	33.23	0.94	2.83
W1–2	⅞	74	216	37.72	0.80	2.12
W1–3	2⅝	390	202	38.73	0.36	0.93
W2–1	⅛	71	193	31.53	1.03	3.27
W2–2	⅞	1	38	37.48	0.95	2.53
W2–3	2⅝	410	228	38.87	0.42	1.08
W3–1	⅛	77	30	29.82	0.53	1.78
W3–2	⅞	440	43	36.05	0.65	1.80
W3–3	2⅝	610	21	37.38	0.69	1.85
W4–1	⅛	2	11	28.81	0.71	2.46
W4–2	⅞	17	33	35.91	0.89	2.48
W4–3	2⅝	107	19	38.39	0.86	2.24

[a] Counts expressed as multiples of one million.

creased in number from the interior portions of the cheese to the exterior. A minor exception to this pattern occurred upon examination of Cheese W2. Position 3 of Cheese W2 contained 410,000,000 propionibacteria per gram. Position 2, which was 1½ in. nearer the surface, contained less than 1,000,000 propionibacteria per gram. Position 1, however, located ½-in. nearer the surface than Position 2, and only ⅛-in. under the surface of the cheese, yielded 71,000,000 propionibacteria per gram.

Propionibacteria in Cheese B11 decreased from 1,350,000,000 per gram in Position 3 to 1,000,000 per gram in Position 1. Cheese B12, which was made on the same day and was cured simultaneously with Cheese B11, contained 2,170,000,000 propionibacteria per gram in the interior of the cheese (Position 3). Position 1 of this same cheese contained less than 1,000,000 propionibacteria per gram. Another example of this decrease in propionibacteria existed in Cheese W1. Position 3 in this instance contained 390,000,000 propionibacteria per gram; in Position 1, less than 1,000,000 per gram were present. Cheeses W3 and W4, which had been aged for 16 mo., again showed this typical distribution of propionibacteria. Cheese W3 contained 610,000,000 propionibacteria per gram at Position 3 in the interior of the cheese; Position 1 of this same cheese yielded 77,000,000 per gram. Cheese W4 at Position 3 had 107,000,000 propionibacteria per gram, whereas Position 1 yielded only 2,000,000 per gram.

As has been shown, propionibacteria are not distributed evenly throughout conventional block or wheel-shaped Swiss cheese. Samples taken 2⅝ in. below the surface of six different Swiss cheeses were, in general, quite high in numbers of propionibacteria. Proceeding outward, the number of propionibacteria decreased strikingly. All of the six samples taken ⅞-in. under the surface contained

fewer propionibacteria than were present in the sample areas closer to the center of the cheeses. The decrease in numbers of these organisms was most noticeable in the comparison between the samples taken 7/8- and 1/8-in. under the cheese surfaces.

Considering other types of bacteria, that is, those capable of growing aerobically on carrot-liver infusion agar, this pattern did not exist. Three of the six cheeses did not show any significant decrease in bacterial population of this type from the interior to the exterior of the cheese. In addition, organisms from the aerobic plates were cultured in litmus milk and were examined microscopically. There was no significant variation in numbers or types of these organisms throughout the six different cheeses. As would be expected, streptococci and lactobacilli predominated.

Results of the chemical analysis of the cheese for moisture and salt percentage were normal. In every instance, the interior portions of the cheese were higher in moisture content than the exterior portions. Considering salt content, the reverse of this was true, depending upon the age of the cheese. With cheese 2 mo. and younger, the salt was concentrated in the outer portions of the cheese. The salt in Samples W3 and W4, 16 mo. old, was distributed evenly throughout the cheese. When expressed as the per cent of salt in the moisture present at the different sample locations, this uniformity of salt distribution throughout Cheeses W3 and W4 becomes even more apparent.

DISCUSSION

The great variation in numbers of propionibacteria at the different sample locations is undoubtedly the result of several inseparable factors. Propionibacteria are characterized by their general inability to grow aerobically unless present in large numbers. During the earlier stages of Swiss cheese making, relatively few propionibacteria are present in the curd. It is during this time that the concentration of salt in the outer portions of the wheel is at its highest level. In addition, the outer portion of the curd mass at this time is low in moisture. High concentrations of salt in the moisture phase would inhibit growth of propionibacteria, especially if the oxidation-reduction potential were unfavorable.

At the same time, conditions at the surface of the cheese are at the most unfavorable, that is, during the early part of the warm-room treatment, conditions in the interior of the cheese would be most favorable for the growth of propionibacteria. Here, the curd would have a low oxidation-reduction potential, as has been determined in this laboratory. Moisture content would be comparatively high and salt content would be low. The temperature at this point is selected to favor the growth of the organisms (72–78° F.). Consequently, under these favorable conditions the propionibacteria in the interior of the cheese are able to increase greatly. At this same time, the same organisms in the exterior portions of the cheese are growing more slowly. This creates the great disparity in the specific bacterial population. After the warm-room treatment, the cheese is held at a temperature range (40–45° F.) not conducive to growth of propioni-

bacteria. As a result, the difference in numbers would have little chance to equilibrate itself.

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INFLUENCE OF PASTEURIZATION AND COOL-AGING ON THE BEHAVIOR OF PURE SALT SOLUTIONS PREPARED IN ACCORDANCE WITH THE COMPOSITION AND CONCENTRATIONS IN MILK

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SUMMARY

A pure salt solution was prepared to simulate the composition of salts in milk serum, in order to study the mineral balance in milk. The salts were distributed into soluble and colloidal phases and a sediment was separated from the solution by centrifugation. Storage resulted in formations that resembled "lime grains." Analysis showed that 35.9, 72.9, 54.8, and 80.9% of Ca, Mg, P, and citric acid, respectively, were soluble. The Ca:P ratio in the sediment was 1.46, indicating predominance of tricalcium phosphate.

Heating decreased soluble Ca and pH, whereas cool-aging had the reverse effect. Variations in soluble Mg and citric acid were insignificant, but soluble P decreased on heating and on cool-aging in the pasteurized lot. The Ca:P ratio in the sediment from the untreated aliquot decreased to 1.26 on cool-aging, but pasteurization increased it to 1.63, which decreased again to 1.08 on cool-aging. Adding Ca increased Ca:P ratio, whereas adding P decreased it. The pH decreased with added Ca and increased with added P. Undissolved Ca, Mg, and citric acid in the sediment, when accounted for as tribasic salts, left 0.5 mg. of P and 0.4 mg. of Mg unaccounted for per 100 ml. of solution.

In attempting to explain the various phenonema resulting in alterations in the ionic equilibria in milk under various processing conditions, resemblance with the behavior of pure salts is often sought and indicated. Such comparisons have been common in explaining: the influence of heat treatments and sterilizatioin on pH of milk; variations in rennet-coagulation time relative to heat treatment and temperature history; effect of addition of salts as correction in evaporated milk, and other phenomena. The alterations in pH of milk on heating have been explained in terms of shifts of tricalcium phosphate equilibrium *(6, 7, 8, 9)*, as is rennet hysteresis, which also is explained, in part, as being related to the ionic equilibria in milk. Although many explanations appear to be reasonable, it is obvious that observations on the mineral balance in milk should be interpreted with a consideration of the proteins, lactose, fat, and other minor constituents present. Studies on simplified systems of various types have been made to determine the extent to which these interpretations are justified, as well as to verify the observations made on milk *(3, 4, 11, 12, 13)*. The present study was undertaken to see if changes in pH and salt balance found under various processing treatments in milk can be observed and verified under similar conditions, in mixtures of pure salts prepared to simulate the composition of salts in milk.

Zoller *(14)* reported studies on salt solution, where he followed the precipi-

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tation of calcium quantitatively and measured the variations in pH resulting from heat treatment. He reported a progressive loss of calcium from the solution with increasing intensity of heat. This was also accompanied by a proportionate increase in the hydrogen ion concentration. The data on the composition of the salt solution, and other analytical observations, were not reported.

In the present study, an attempt to arrive at a salt solution was based on the synthetic milk studies of Clark *(1)*. On elimination of proteins and sulfuric acid a mixture was obtained, but the pH was much higher than is usual for normal milk. After several trials, the following combination of salts was found satisfactory:

	(per liter)
$CaCl_2 \cdot 2H_2O$	2.62 g.
MgO	0.31 g.
KH_2PO_4	3.26 g.
Citric Acid $\cdot H_2O$	2.06 g.
NaOH solution (1 *N*)	21.0 ml.
KOH solution (0.97 *N*)	10.0 ml.
$Ca(OH)_2$ (0.1 g. Ca/100 ml.)	150.0 ml.

The expected final composition in grams per liter was: calcium, 0.87, magnesium, 0.19, phosphorus, 0.74, citric acid, 1.88, sodium, 0.48, potassium, 1.32, and chlorine, 1.27.

In arriving at the level of calcium in this solution, the amount of calcium known to be in combination with casein was deducted from the total calcium in milk. In like manner, the amount of phosphorus known to exist in combination with casein also was discounted. Based on the data of Van Slyke and Bosworth *(7)*, 1.63% P_2O_5, and according to de KaKdt and van Minnen *(5)*, 1.5% CaO, were deducted, in arriving at the composition of the solution.

It may be pointed out that the final composition of this salt solution is considerably different from the composition of salts in the synthetic milk of Clark. After discounting the amounts of minerals combined with proteins, as indicated, the composition can be regarded to fall within the normal range for calcium, magnesium, phosphorus, and citric acid in normal milk. Further, the di- and trivalent ions were the primary concern of this study and, since the influence of the monovalent ions is relatively small, these were not controlled to the same extent as the former. This became necessary and incidental to achieving a desirable pH, which was obtained by using NaOH.

The MgO was dissolved in 500 ml. of water. Then the calcium chloride, dissolved in 50 ml. of water, was added and then citric acid. This solution had a pH of 3.6. While stirring with a mechanical stirrer, 10 ml. of KOH, followed by 15 ml. of NaOH, were added. The potassium phosphate was dissolved in 200 ml. of water and added very slowly from a burette (with vigorous stirring). About 150 ml. of calcium hydroxide were then added dropwise and the pH adjusted to 6.7 with an additional 6.0 ml. of NaOH. The solution obtained was made to one liter with water and held about 10 hr. in a refrigerator at

45° F. The pH decreased to 6.67. This solution was used in further study, which is described. Any treatments given were in addition to this holding period of 10 hr. The solution was warmed to room temperature and the actual amounts of calcium, magnesium, phosphorus, and citric acid were determined analytically. The solution showed some evidence of grain formation in an aliquot which was allowed to stand in cold for from three to four days. The grains resembled the typical lime grains that occur in evaporated milk.

A viscous and colloidal sediment separated upon centrifugation of the solution at room temperature for 15 min. at 1,500 r.p.m. The supernatant was analyzed for soluble salts. The analyses revealing the distribution of calcium, magnesium, phosphorus, and citric acid are given (Table 1). The lack of agreement between the expected amounts of calcium, magnesium, phosphorus, and citric acid and the amounts found on actual analyses were owing to approximations involved in weighing and also reflect impurities in the reagents.

The effects of pasteurization and cool-aging on the distribution of these salts into sediment and supernatant also were studied. The temperature of a 250-ml. aliquot of the solution was increased to 143° F. in a water bath with constant agitation and maintained for 30 min. It was then cooled and transferred to a 250-ml. volumetric flask and the volume restored again with distilled water. These results also are presented (Table 1). Results of the influence of added calcium and phosphorus salts under these conditions are given (Table 2). Significant differences were found in the pH of the solution under the various treatments and are reported (Table 3).

DISCUSSION

It was indicated by Sommer and Menos *(8)* that the calcium and phosphorus in milk exceeds the solubility product of tricalcium phosphate. Therefore, milk contains precipitated tricalcium phosphate in a finely divided form. In the pure salt solution, also, the precipitate was found to be colloidal. The colloidal phase, however, did not stay dispersed in the salt mixture, and the proteins in milk maintained the dispersion because of their protective effects.

Tricalcium phosphate precipitation does not occur instantaneously on heating, but several hours are required for equilibrium. This fact was supported by the behavior of the pure salt solution. With a decrease in the amount of soluble

TABLE 1

Influence of heating and cool-aging on the dstiribution of salts in the supernatant and centrifugal sediment and on pH and Ca:P ratios

	Calcium	Magnesium	Phosphorus	Citric Acid	pH	Ca:P ratios in sediment
	(mg/100 ml.)					
1. Total	94.0	19.2	75.5	155.1	6.67	.....
2. Supernatant						
a. In untreated	33.8	14.0	44.1	125.5	6.67	1.46
b. In (a) after 24 hr.	30.0	15.7	37.3	130.6	6.39	1.26
c. In pasteurized	23.7	17.0	39.2	131.2	6.28	1.63
d. In (c) after 24 hr.	29.4	17.3	32.5	131.7	6.32	1.08

TABLE 2

Influence of added calcium and phosphorus on the distribution of calcium, magnesium, and phosphorus in pure salt solutions and on the pH and Ca:P ratios

Treatments	Calcium	Magnesium	Phosphorus	pH	Ca:P ratios in sediment
	(mg./100 ml.)				
1. Total in sample	94.0	19.2	75.5	.....	.....
2. Total in sample with added P[a]	.....	.....	97.5	.....	.....
3. Total in sample with added Ca[a]	139.1	.....	.....	.....	.....
4. Soluble in untreated	33.8	14.0	44.7	6.67	1.48
5. Soluble in pasteurized	23.7	17.0	39.2	6.28	1.71
6. Soluble in pasteurized and cool-aged	29.4	17.3	32.5	6.32	1.16
7. Soluble in pasteurized after addition of phosphorus	17.2	15.0	45.3	6.65	1.14
8. Soluble in above after cool-aging	21.0	16.7	47.5	6.55	1.13
9. Soluble in pasteurized after addition of calcium	45.0	15.7	31.0	5.63	1.63
10. Soluble in above after cool-aging	50.5	11.9	25.6	5.25	1.37

[a] 22 mg. per 100 ml. phosphorus added as Na_2HPO_4, and 45 mg. per 100 ml. calcium as calcium acetate.

calcium, there was a simultaneous decrease in the pH, and vice versa. The variations in pH were the result of tricalcium phosphate precipitation. Magnesium phosphate precipitation must also be considered, because the solubility of this salt is different from that of tricalcium phosphate at higher temperatures.

On aging, there was only a slight increase in soluble magnesium in the untreated aliquot, whereas the pasteurized and aged portion showed even less increase in this fraction of magnesium. However, there was a decrease in the solubility of phosphorus from raw to pasteurized, which showed further decrease on cool-aging. This may indicate a reversion of tricalcium to dicalcium phosphate.

The behavior of citrate in milk is of interest. The prevention of clotting of milk with rennin by adding citrate is often regarded as resulting from the formation of un-ionized calcium citrate, which removes calcium ions from solution, these ions being considered essential for the clotting process. But Wiley (*10*) states that more citrate is present than is necessary for equivalence with calcium. Thus, if un-ionized tricalcium citrate were formed, there would

TABLE 3

Distribution of added calcium and phosphorus in the salt solution after various treatments

	Calcium	Phosphorus
	(mg. %)	
1. Added in 100 ml. of solution	45.0	22.0
2. Recovered in the supernatant from:		
(a) Pasteurized sample	21.3	6.1
Per cent of total	47.3	27.7
(b) Pasteurized and aged sample	21.1	15.0
Per cent of total	46.8	68.1

be no calcium ions in normal milk, and if calcium ions were necessary for coagulation, milk would not clot. Somehow, citric acid does not form much tricalcium citrate in milk; otherwise, based on its dissociation properties there would be practically no ionized calcium in milk. The true salt mixture also did not form much tricalcium citrate, and in this respect the behavior of the salt mixture and of the milk was alike. The effect of ionic concentration may be responsible in some way for the lack of formation of tricalcium citrate in salt mixtures. In milk, the situation is further complicated by the presence of other organic constituents. Another similarity in the nature of citrate in milk with salt mixture may be pointed out. Contrary to earlier reports *(2, 7)*, the citrate in milk is present in colloidal form to the extent of about 15% in rennet curd *(9)*, which also is likely to be the case in milk without rennet treatment. This also holds true for pure salt mixture.

The mole ratios of Ca:P in the insoluble phase of the salt solution were computed according to Verma and Sommer *(9)*. The variations in these ratios may indicate shifts of equilibria, rather than precise evidence of the exact salt combination. Thus, we see (Table 1) that the sediment in the untreated salt mixture had a Ca:P ratio of 1.46, which changed to 1.26 on cool-aging. Pasteurization increased the ratio to 1.63, which also decreased to 1.08 on cool-aging. These changes may be explained in terms of tricalcium phosphate precipitation on pasteurization and reversion to dicalcium phosphate on cool-aging. The pH of the mixture was reduced from 6.67 to 6.28 on pasteurization, with an increase to 6.32 on cool-aging. Qualitatively, this also was in harmony with the changes in tricalcium phosphate precipitation indicated from Ca:P ratios above.

It is seen (Table 2) that the pasteurized aliquot to which calcium was added had a Ca:P ratio of 1.63 as compared to 1.48 for the untreated control and 1.71 for pasteurized. In this table, the Ca:P ratios are calculated without the benefit of data oin citric acid, because these analyses were not included here. These ratios may not be compared to the figures in Table 1, except in a qualitative sense. The ratio in calcium-added milk decreased to 1.37 on cool-aging. The addition of calcium decreased the pH from 6.28 to 5.63 with further decrease during cool-aging. Phosphorus addition caused an increase in pH from 6.28 to 6.65, which decreased on cool-aging.

Increase of calcium and pasteurization both caused a decrease in the pH, but cool-aging did not increase the pH in the calcium-added aliquot within the period of 24 hr. It probably takes longer to aproach equilibrium. Phosphate-added aliquot showed lower Ca:P ratio and increased the pH. This is consistent with dicalcium phosphate formation. Cool-aging decreased Ca:P ratio in the sediment, but the decrease in the pH in this case was not much.

The distribution of the added salts into the supernatant and sediment as a result of the various treatments was computed and is shown (Table 3). The fate of the added calcium and phosphorus in the salt solution showed significant variation. Whereas the amount of calcium in supernatant from pasteurized and cool-aged treatments did not vary, the phosphorus showed significant differ-

ences—27.7 and 68.1%, respectively—for the two treatments. This also confirmed insoluble phosphate formation on pasteurization, which reverts to soluble phosphate on cool-aging.

It can also be seen (Table 1) that in 100 ml. of salt solution the amounts of different salts that remained undissolved upon centrifugation were: calcium, 60.2 mg., magnesium, 5.2 mg., phosphorus, 31.4 mg., and citric acid, 29.6 mg. If all the calcium in this were to form tricalcium phosphate, 60.2 mg. Ca would require 30.9 mg. P, leaving a remainder of only 0.5 mg. of P. Also, assuming that all the citric acid combined with Mg to form Mg-citrate, 29.6 mg. of citric acid would require 5.6 mg. of Mg, leaving a deficit of only 0.4 mg. of Mg. Thus, the salts remaining undissolved in the mixture made to simulate salts in milk are in substantial balance, on the basis of their occurrence as tribasic phosphates and/or citrates of calcium and/or magnesium. The influence of pasteurization and cool-aging of pasteurized aliquot, and of addition of calcium and phosphorus, also are understandable in terms of shifts of ionic equilibria. Thus, the deviations in the behavior of salts in milk from this pattern should be explained in terms of the influence of the other colloidal systems present in milk.

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USE OF FLUORESCENT MATERIALS FOR THE INDIRECT DETECTION OF ANTIBIOTICS IN MILK

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SUMMARY

The addition of a combination of fat-soluble fluorescein (Fluoral) and uranine, as a "marker," to penicillin preparations intended for intramammary infusion, was found to provide a rapid and satisfactory means for detecting the antibiotic in milk. The marker was detected visually in the milk for 48 hr. after treatment and with ultraviolet light for 96 hr. The marker was nontoxic to the treated animals and did not affect milk production. Statistical analysis of the data showed a close correlation between the excretion of marker and penicillin from the treated udders.

A rapid, sensitive, and reliable means for detecting antibiotics in milk is needed urgently by dairy manufacturers, dairy farmers, and public health officials. Although several sensitive and apparently reliable tests for antibiotics have been proposed recently, all of them are laboratory tests that require from 2½ to 8 hr. *(1, 3, 4)*. The time, laboratory equipment, and skill required for these tests reduce their usefulness, particularly to cheese-makers and farmers.

A rapid and practical test for antibiotics in milk would enable farmers to determine definitely how long to withhold the milk from antibiotic-treated cows; for manufacturers to avoid serious economic loss resulting from the injurious effects of antibiotics on bacterial starters used in making cheese and fermented milks, and for public health officials to make routine tests of large numbers of milk samples.

The incidence and concentrations of antibiotics in market milk have been reported recently *(2, 5, 6)*, and cheese-makers have frequently encountered complete failure of bacterial starters, owing to the presence of antibiotics in the milk.

In 1950, the Agricultural Research Service encountered sporadic appearances of antibiotics in the milk being used for making experimental cheese. At that time, the feasibility of incorporating a dye in all antibiotic preparations to be used for intramammary infusion was investigated. Such a dye would serve as a visual index of antibiotic contamination in milk. The ideal dye for this purpose would have to remain in the milk as long as the antibiotic, have no effect on the antibiotic activity, and be nontoxic and nonirritating to the cows' udders. Although some of the dyes tested in the early studies were readily detectable visually for 48 hr. after their infusion into udders, they failed to remain in the milk as long as did penicillin.

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Recently, these studies have been resumed, using fluorescent materials and additional dyes as antibiotic markers. Although several phases of the work remain to be completed, preliminary experiments indicate that it is feasible to add such markers to antibiotics intended for veterinary use. This report briefly presents the pertinent findings.

EXPERIMENTAL PROCEDURE

Various chemicals were screened, to find an agent that could be used as a tracer, or marker. Selection was based primarily upon fluorescence, color, and odor. The list of chemicals tested included certified food colors, cosmetic dyes, food-flavoring compounds such as vanillin, cinnamaldehyde, and methyl anthranilate, odoriferous materials such as furfuryl mercaptan and gamma undecalactone, and various fluorescing agents such as the fluoresceins, chlorophylls, and hydroxycoumarins. In the preliminary study, each material was tested for ease and limits of detection in whole milk. Milks containing fluorescent materials were diluted serially and measured for fluorescence with a 2-amp. long-wave ultraviolet (3660Å) lamp. Effects of the chemical upon the antibiotic activity of penicillin were determined by penicillin assays with *Streptococcus thermophilus*. Materials that appeared to have value as tracers were tested further, for their possible effects on antibiotic activity during storage. In most instances, mixtures of the chemical and penicillin in mineral oil and also in a water-in-oil preparation (Penicle),[2, 3] were stored at room temperature. The limits of detection for tracer agents were established visually by a number of individuals, both under normal and under ultraviolet light. Agents selected on the basis of the in vitro tests were subjected to further tests, by injection into udders, either alone or in combination with penicillin.

Three separate trials were conducted to evaluate the marker materials in the in vivo studies. Some markers were subjected to additional trials if further testing seemed advisable. Each trial was designed as a balanced incomplete block experiment, with four marker treatments assigned at random to the four quarters of the udder of each of six cows representing a wide range in milk production. A common source of oil-penicillin preparation was used in preparing the emulsions of tracer materials. In every instance, one 15-ml. dose, containing known amounts of marker, oil, and penicillin, was administered. The dose of penicillin ranged from 100,000 to 300,000 units, and the dose of tracer from 250 to 400 mg. Quarter-milkers were used to collect samples of milk from the individual quarters for at least 96 hr. after infusion. Quarter-milkings prior to treatment were used as controls for pH measurements and leucocyte counts. Close veterinary scrutiny and complete milk production records were maintained. Milk samples from the treated quarters were tested for leucocytes, pH, penicillin content, and for the presence of marker. Further, milks containing marker

[2] Pitman and Moore.

[3] The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

were diluted with normal herd milk to determine the extent of dilution without loss of marker detection.

Trial I. 4-Methyl umbelliferone, methyl anthranilate, esculin, and uranine (sodium fluorescein) were used as marker materials. Each test dose contained 250 mg. of marker and 100,000 units of penicillin G in 15 ml. of an oil base containing equal parts of light mineral oil and petrolatum.

Trial II. Uranine, oil-soluble fluorescein (Fluoral 7 Ga.)[3, 4] a combination of uranine and oil-soluble fluorescein, and spirit-soluble fluorescein were compared as marker materials. Each dose contained 250 mg. of marker and 100,000 units of penicillin in an oil base. The oil base in this trial was Penicle.

Trial III. Esculin, a combination of esculin and Amaranth Red No. 2, oil-soluble fluorescein, and oil-soluble chlorophyll were tested. Each test dose contained 400 mg. of marker and 300,000 units of penicillin G in 15 ml. of Penicle.

Data accumulated from these trials were statistically analyzed to evaluate the marker materials, as well as to determine the effect of the level of milk production on the rate of excretion of the antibiotic and marker from the udder.

RESULTS

The dyes tested to date do not seem to have as much potential as tracer materials as do the fluorescing agents. For the most part, the odorous or flavored materials were unsatisfactory as tracer agents. The limits of identification for these materials varied tremendously from one individual to another, and to a lesser degree with milk quality. In this report, only the marker materials that have been tested by udder infusion will be considered.

Limiting concentrations of marker material at which color of fluorescence was apparent in milk are shown (Table 1). Laboratory attempts to isolate tracer materials from the milks were successful with a few agents, but such procedures were not considered to be practical for routine identification. The fluorescein derivatives could be detected in milk both by color and by fluorescence in rather high dilution. As may be noted, esculin and 4-methyl umbelliferone were detected in milk only by the use of ultraviolet light. Methyl anthranilate was detected by ultraviolet light and also by its grape-like odor and taste.

None of the markers caused a degradation in the antibiotic activity of penicillin stored in mineral oil at room temperature over a period of 4 mo. In the

[4] General Dyestuff Company.

TABLE 1

Limiting concentration of marker detected in whole milk

Marker	Ordinary light	Ultraviolet light
	(p.p.m)	
Esculin	Not detectable	1
Uranine	200	10
Oil-soluble fluorescein	200	10
Spirit-soluble fluorescein	100	10
4-Methyl umbelliferone	Not detectable	1
Methyl anthranilate	Not detectable	10
Amaranth Red No. 2	100	Nonfluorescent
Oil-soluble chlorophyll	200	200

Penicle preparation stored at room temperature, Amaranth Red caused a marked reduction in penicillin activity. The limits of identification for the markers in milk were not affected by storage with penicillin in any of the oil preparations.

In the in vivo studies, it became evident that some of the materials were unacceptable as tracer agents, whereas others showed considerable promise. Some agents appeared to be slightly toxic and others were absorbed from the udder. Marker agents were evaluated after a statistical analysis of the data from the penicillin and marker determinations on the treated milks from individual cows. The values listed (Table 2) are the averages for the six cows used to test each marker. These data show the number of milkings after treatment during which penicillin was being shed in the milk at different levels, and the number of milkings during which the markers could be detected at various dilutions. In every instance, penicillin was detected at the 72-hr. period and, in many instances, at 96 hr. or longer. This emphasizes the need for a test to detect antibiotics in milks as long as they are excreted, rather than depending on a fixed period of 72 hr. for the milk to be withheld.

Trial I. The persistency of the marker materials in the udder did not appear statistically different. However, esculin and uranine were definitely superior with respect to detectability in the treated milks. The veterinary report indicated clearly that 4-methyl umbelliferone and methyl anthranilate were unsuitable as marker agents. 4-Methyl umbelliferone caused a slight but definite irritation in all the test quarters, whereas methyl anthranilate, although nontoxic, partially was absorbed in the udder and appeared in the urine of the cows. No rise in leucocyte count or pH of the milk was noted either with esculin or with uranine.

Trial II. In this trial, the marker persistency at the zero and 1 : 4 dilutions showed a highly significant difference between markers. Oil-soluble fluorescein had the greatest persistency and was readily detected in the herd milk. The leucocyte count and pH of treated milks remained constant.

Trial III. In Trial III, marker persistency at the zero and 1:4 dilutions showed highly significant differences between markers. However, the differences between esculin, a combination of esculin and Amaranth Red, and oil-soluble fluorescein were not significant. Chlorophyll was definitely inferior to the three other test materials. (Results of recent tests with increased doses of chlorophyll suggest that it has much greater value as a marker than is indicated in Table 2.)

The combination of uranine and oil-soluble fluorescein was very effective as a marker, in that it definitely colored the milk for at least 48 hr. after infusion, and was detectable by ultraviolet light for 96 hr. after infusion.

The retention of penicillin and marker in the quarter was influenced greatly by the quantity of milk being produced. Low producers retained penicillin and marker in the quarter as much as 24 to 48 hr. longer, and at higher levels, than did the higher producers.

Increasing the penicillin dose from 100,000 to 300,000 units had little effect upon the number of milkings in which penicillin persisted. However, a greater proportion of penicillin appeared in the earlier milkings with the larger dose.

TABLE 2

Persistence of penicillin and markers in milk

Trial	Marker	Marker dose	Penicillin dose	Av. milk yield per quarter	Penicillin detected at levels of: 0.1	0.2	1.0 (units/ml)	Marker detected at dilutions of: 0	1–4	1–9
		(mg.)	*(units)*	*(lb.)*	*(Av. No. milkings)*					
I	1. 4-Methyl umbelliferone	250	100,000	.....	6.25	5.34	3.22	2.05	0.55	0.02
	2. Methylanthranilate	250	100,000	.....	7.79	6.71	4.18	1.31	0.19	0
	3. Esculin	250	100,000	.....	7.01	6.06	3.86	9.10	5.88	3.77
	4. Uranine	250	100,000	.....	7.17	5.88	3.22	10.24	6.03	3.35
II	1. Uranine	250	100,000	3.01	8.24	7.24	4.90	2.73	1.58	0.92
	2. Oil-soluble fluorescein	250	100,000	3.41	7.34	6.50	4.37	8.70	5.36	3.16
	3. Combination uranine and oil-soluble fluorescein	125 (each)	100,000	4.00	8.00	7.06	4.73	5.51	3.73	2.55
	4. Spirit-soluble fluorescein	250	100,000	3.92	7.44	6.54	4.45	4.57	3.15	2.20
III	1. Esculin	400	300,000	3.88	7.15	6.45	4.81	7.69	5.96	4.81
	2. Combination, Amaranth Red and esculin	400 (each)	300,000	3.71	7.24	6.50	4.77	7.54	5.88	4.78
	3. Oil-soluble fluorescein	400	300,000	3.71	7.18	6.48	4.84	7.96	5.47	3.82
	4. Oil-soluble chlorophyll	400	300,000	3.78	8.03	7.21	5.30	2.94	0	0

Increasing the marker doses had a similar effect, especially with the water-soluble agents. The oil-soluble materials appeared to remain in the udder slightly longer and at a higher concentration level than did most of the water-soluble agents.

Table 3 shows the correlation coefficients between penicillin and marker persistency. The correlation was obtained for the over-all milkings as well as for the within-milkings. It is of interest to note that a combination of uranine and oil-soluble fluorescein was more closely associated with penicillin persistency than any other marker, or combination of markers.

In Trial II, an increase of 1 lb. of milk yield decreased penicillin persistency (at a level of 0.10 penicillin unit) by 0.28 milking. In Trial III, an increase of 1 lb. of milk yield decreased penicillin persistency by 0.115 milking. The fact that milk yield had less effect on penicillin persistency in Trial III might be attributed to the fact that dosage of penicillin administered in Trial III was much greater than in Trial II.

In Trial II, the statistical analysis of data showed that an increase in 1 lb. of milk yield decreased marker persistency at the 1:4 dilution level by 0.31 milking. In Trial III, this decrease was 0.145 milking. Again, the dosage of the marker was greater in Trial III than in Trial II. It is of interest to note the strong parallel in the influence of milk yield on the persistence both of penicillin and of marker.

TABLE 3

Correlation coefficients between penicillin and marker persistency

Trial	Marker	Milkings	
		Total	Within
I	4-Methyl umbelliferone	0.67	0.07
	Methyl anthranilate	0.22	−0.10
	Esculin	0.91	0.84
	Uranine	0.90	0.85
II	Uranine	0.75	0.45
	Oil-soluble fluorescein	0.81	0.61
	Combination of oil-soluble fluorescein and uranine	0.94	0.82[a]
	Spirit-soluble fluorescein	0.85	0.22
III	Esculin	0.88	0.26
	Combination of Amaranth Red and esculin	0.86	0.20
	Oil-soluble fluorescein	0.88	0.34
	Oil-soluble chlorophyll	0.31	−0.19

[a] Perhaps this indicates that a combination of 250 mg. of oil-soluble fluorescein and 125 mg. of uranine is most closely associated with penicillin persistency.

DISCUSSION

Hydroxycoumarin derivatives such as esculin, umbelliferone, and 4-methyl umbelliferone are known for their fluorescence in ultraviolet light. Small quantities were easily detected in milk by their fluorescence. Esculin has been reported to be detectable in water at a concentration of 0.1 p.p.m., and we found it to be strongly fluorescent in milk.

The optimal pH for fluorescence was considered for all materials. The fluorescence of 4-methyl unbelliferone in milk was increased tenfold by adjusting the

milk pH to 9.0. The brilliant blue fluorescence of esculin was more difficult to detect in old milk, as there was a tendency for the milk, as it aged, to change from a greenish to a blue fluorescence. Methyl anthranilate was selected as a possible tracer agent, because it can be added to foods, has a strong grape-like aroma and flavor and, in addition, is fluorescent in ultraviolet light. Although methyl anthranilate and 4-methyl umbelliferone could be extracted in *n*-butyl alcohol, they proved to be unsatisfactory in other respects in the in vivo studies.

Plant chlorophyll is known to produce a fiery-red fluorescence and, as it has been reported to have some medicinal properties, it seemed an excellent material to include in the study. Of the chlorophyll preparations tested, the oil-soluble form showed more potential as a marker. Small quantities in milk could be detected by extraction in *n*-butyl alcohol.

Fluorescein derivatives, as might be expected, gave a bright-green fluorescence and color to the milk. Some difficulty may be encountered in differentiating the yellow fluorescence of uranine from that of normal milk in concentrations of 1 p.p.m. or less. However, adjustment of the milk to pH 7.5 to 8.0 aids in the detection of uranine by diminishing the normal fluorescence of milk. Oil-soluble fluorescein appeared in the treated milks attached to the fat globule. The floating fluorescent globules were easily detected in the fluid milk by ultraviolet light.

In our studies, no evidence of toxicity was noted when esculin, chlorophyll, uranine, and oil-soluble fluorescein were injected into the udders of cows. A lack of toxic effects of these agents when ingested by animals has not been determined in this study. The cost of marker material is an important factor in considering it for commercial use. The amount of oil-soluble fluorescein and uranine added to the veterinary antibiotic preparation should not increase the cost more than one-half cent per dose.

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USE OF ANNATTO FOR COLORING BUTTER[1]

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SUMMARY

A comparison was made of the effect of annatto and coal-tar color on the chemical quality of stored butter. Commercial conditions were duplicated by selecting two levels of cream quality, two levels of copper, and two storage treatments.

Flavor was evaluated by official graders and by a panel of 15 persons. The surface color was determined with a photoelectric reflection meter and also with Dyeco color standards. Peroxide values were determined by the ferric thiocyanate method; aldehyde values by the rosaniline method; acid degrees by titration; copper content by the carbamate method, and vitamin A by the Carr-Price method.

Official graders and a taste panel agreed on a difference in flavor between high- and low-copper samples. The flavor of the high-copper samples decreased on storage. No flavor difference was attributable to the type of color. Peroxide and aldehyde values increased on storage in the samples with high copper content, but the type of coloring agent was not a factor. Acid-degree values increased slightly on storage, but were not affected by the type of color or by the level of copper. Carotene accounted for 10% of the vitamin A potency of the butter; it was not affected by storage for 6 mo. at −5° F., but decreased following additional storage for 2 wk. at 60° F. The preformed vitamin A content of the butter was not affected by the type of color, copper level, or storage.

Annatto color has been used in butter since the early days of the butter industry. During the last few decades, in the United States and Canada, it has been largely replaced by coal-tar dyes, although there are countries where annatto color has remained the main butter color.

For several years the color of American and Canadian butter has been standardized almost exclusively with the coal-tar dyes Yellow AB and Yellow OB. The alleged toxic properties of these dyes and the need for other coloring agents for butter have been discussed in a previous paper *(13)*. The pharmacological aspects of annatto color have only recently received consideration. Engelbreth-Holm and Iversen *(4)* indicated that the vegetable annatto butter color can not be considered cancrogenic to rats and mice. Notwithstanding the extensive use of annatto in butter, the chemical effect of this color on the quality of stored butter has never been studied comprehensively. This was the object of the investigation described in this paper.

EXPERIMENTAL PROCEDURE

Manufacturing. The factorial experiment involved two types of color, two levels of cream quality, two levels of copper, and three storage treatments. The butter was made in April, 1956, from split vats of Special Grade and First Grade cream *(1)*. The Special Grade cream was obtained from the Experimental Farm Dairy. Its initial acidity was uniformly 0.14%. The First Grade cream,

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obtained from a local milk plant, had an initial acidity of 0.20 ± 0.02%, and a reasonably clean flavor. The fat content of the cream was 32% in each vat. The acidity was standardized to 0.10% acidity with soda neutralizers. Lots of cream from two sources were pasteurized separately in a vat of 3,000-lb. capacity at 170° F. for 10 min., then cooled to and maintained at 45° F. overnight. Each vat of cream was split into four lots, the respective lots being treated with *(a)* annatto color; *(b)* annatto color plus 3 p.p.m. copper; *(c)* coal-tar color, and *(d)* coal-tar color plus 3 p.p.m. copper.

Coal-tar color (Hansen) was added at the rate of 1 oz. per 100 lb. of fat in 12 lots of cream. To approach the color of the butter colored with coal-tar, annatto color (Hansen) was added to six comparable lots of cream at the rate of 3 oz. per 100 lb. of fat, and to six other lots at 4 oz. per 100 lb. of fat. It was assumed that this difference in the level of annatto color had no effect on the butter other than a difference in color intensity. All annatto-colored samples were treated as one group, for the analysis of variance of all values except color values.

Copper was added as a 0.5% solution of copper sulfate, at the rate of 3 p.p.m. of the cream, in 12 comparable lots involving the two types of color and the two levels of cream quality. This addition raised the copper content of the resulting butter from 0.04 to 0.70 p.p.m.

Annatto color, coal-tar color, and copper were added to the cream in the churn. The cream was churned at 53° F. The butter was salted at the 2.0% level.

Samples from each churning were examined initially. Two 7-lb. blocks of butter from each churning were wrapped in parchment paper, boxed, and stored. One of the two stored samples from each churning was examined after 6 mo. at −5° F., and the other, after 6 mo. at −5° F. plus 2 wk. at 60° F.

Methods of analysis. Three government graders scored the samples for flavor and evaluated the color by comparison with Dyeco color standards.[2] Each set of four comparable butters was ranked for flavor from one to four, in decreasing order of preference, by a taste panel of 15 persons.

Butter oil was prepared by melting and filtering the butter at 122° F. just before analysis for peroxide, aldehyde, acid-degree, and color values. Peroxide values were determined by the method of Lips *et al. (10)*. Schibsted's method *(14)*, as modified by Mummery *(12)* and Dolby *(2)*, was used for aldehyde values. The latter were expressed in terms of density value × 100, at 510 mμ, using a 1-cm. cell and a 5% fat solution. The fat acid degrees were expressed in ml. of 0.050 *N* sodium ethylate per 5 g. of fat. They were determined by titrating 5 g. of the butter oil in 50 ml. of 1 : 1 benzene-ethanol to the phenolphthalein end-point with 0.0040 *N* sodium ethylate. The colors of the butter oils were objectively determined by measuring the optical density at 450 mμ of a 20% solution of the oil in benzin. The color values were reported as γ β-carotene per g. of fat; the extinction coefficient was $\Sigma_1^{1\%/ml}_{cm.} = 0.270$.

The surface color of the butter was determined objectively with the use of a Photovolt photoelectric reflection meter, Model 610. The tristimulus values and

[2] Dye and Chemical Co. of Canada Ltd., Kingston, Ontario, Canada.

the Hunter *a*, *b*, and *L* values were calculated according to equations given by Judd *(7)*. The copper contents of the butter were determined by the method described by McDowell *(11)*. The method of Koehn and Sherman *(9)*, as described by Jenness and Palmer *(6)*, was used to determine the vitamin A potency of the butters.

RESULTS AND DISCUSSION

Color. The average yellow color produced by 3 oz. of annatto color per 100 lb. of fat was equivalent to 6.05 γ of β-carotene per g. of fat, and to a *b* value of 30.08. That of their comparable churnings colored at the rate of 1 oz. of coal-tar color per 100 lb. of fat was equivalent to 7.56 γ of β-carotene per g. of fat, and to a *b* value of 32.30. That these color differences were significant at P = 0.01 is shown in Table 1. The churnings comparing 4 oz. of annatto color and 1 oz. of coal-tar color per 100 lb. of fat were not significantly different in yellow color. It was estimated that 3.7 oz. of annatto color had approximately the coloring power of 1 oz. of coal-tar color.

TABLE 1

Analysis of variance of color values

Source of variation	Degrees of freedom	Mean square			
		As β-carotene	*a* value	*b* value	*L* value
Annatto *(3 oz/100 lb. fat)*					
Color	1	20.44[a]	18.45	44.74[a]	0.24
Copper	1	0.08	0.02	12.82	0.16
Color × copper	1	0.20	0.00	0.14	0.55
Storage	2	2.90	2.35	7.74	0.98
Storage × color	2	0.16	0.02	0.54	0.06
Storage × copper	2	0.22	2.43	4.70	0.05
Error	26	0.99	6.28	3.11	1.08
Annatto *(4 oz/100 lb. fat)*					
Color	1	0.03	22.97[b]	0.14	13.12[a]
Copper	1	1.21[b]	1.35	5.53[b]	0.00
Color × copper	1	1.22[b]	3.21	0.48	0.05
Storage	2	1.13[b]	0.35	3.35	0.02
Storage × color	2	0.21	6.42	0.38	0.50
Storage × copper	2	0.36	2.54	2.05	0.65
Error	26	0.26	3.88	1.05	0.57

[a] Significant at P = 0.01.
[b] Significant at P = 0.05.

The mean *a* values for the comparable churnings colored at the rate of 3 oz. of annatto color, and at the rate of 1 oz. of coal-tar color per 100 lb. of fat were, respectively, −1.71 and −3.14. Those for the comparable churnings colored with 4 oz. of annatto color and with 1 oz. of coal-tar color per 100 lb. of fat were, respectively, −1.46 and −3.06. Higher *a* values indicate more redness, but Table 1 shows that the differences in *a* values were significant only in the latter comparison.

The degree of lightness was similar for the comparable butters colored with 3 oz. of annatto color and with 1 oz. of coal-tar color per 100 lb. of fat. The mean *L* values were 87.99 and 88.99, respectively, for the comparable samples colored with 4 oz. of annatto color and with 1 oz. of coal-tar color per 100 lb. of fat.

Table 1 shows that this difference was significant at P = 0.01.

The butter colored with 1 oz. of coal-tar color per 100 lb. of fat differed by 2.0 and 2.7 NBS units, respectively, from that colored with 3 and 4 oz. of annatto color per 100 lb. of fat.

Copper and storage effected a slight decrease in the degree of yellowness in the group of samples colored with 4 oz. of annatto color per 100 lb. of fat.

Flavor. A taste panel of 15 persons ranked for flavor the comparable butters colored either with annatto or with coal-tar, at two levels of copper. A difference between some of these treatments was indicated by the coefficients of concordance *(8)* significant at P = 0.01 (shown in Table 2). This was corroborated by the analysis of variance of the normal deviates *(5)*. Using the multiple range test of Duncan *(3)*, it was established that this difference concerned only the copper treatment, and that the panel members favored the low-copper samples, except for the coal-tar versus annatto plus copper at initial ranking. In no instance did the taste panel find the flavor to be influenced by the type of color used. That the type of color did not affect the flavor of the butter as scored by official graders is shown (Table 2).

TABLE 2

Effect of type of color on flavor of stored butter

Treatment	Storage		
	Initial	6 mo., −5° F.	6 mo., −5° F. plus 2 wk., 60° F.
Flavor score:			
Chi-square of flavor score	5.07	5.33	2.09
Degrees of freedom	2	3	3
Flavor ranking:			
Av. No. judgments [a]	15	13	13
		Av. sum of rankings[a]	
Annatto	32.2	30.2	21.5
Coal-tar	37.0	27.3	24.7
Annatto plus copper	38.3	36.0	40.3
Coal-tar plus copper	42.5	36.5	43.5
Concordance coefficient	14.94 [b]	5.93 [b]	53.91 [b]

[a] Average of six replicates.
[b] Significant at P = 0.01.

Chemical variables. Table 3 shows that the type of color had no effect on peroxide and aldehyde values. In the samples low in copper content (0.04 p.p.m.), the peroxide and the aldehyde values did not change on storage. High copper, however, increased both values on storage. After 6 mo. at −5° F. plus 2 wk. at 60° F., the peroxide value had increased from 0.39 to 3.16 and the aldehyde value from 3.25 to 60.93. Similar observations were made in a previous study, which compared β-carotene with coal-tar color *(13)*.

The acid-degree values averaged 0.82 in the fat from Special Grade cream and 1.21 in fat from First Grade cream. Storage increased the acid-degree value from 0.95 to 1.07. Table 3 shows that these two differences were significant at P = 0.01. The type of color and the level of copper had no effect on the acid degree value.

Vitamin A potency. In these butters manufactured from winter cream, caro-

TABLE 3

Analysis of variance of peroxide, aldehyde, acid-degree values, and of vitamin A potency

Source of variation	Degrees of freedom	Mean square			
		Peroxide value	Aldehyde value	Acid-degree value	Vitamin A potency
Color	1	0.1398	144.87	0.0018	1.2366
Cream	1	0.0210	218.37	2.7067[a]	0.2901
Copper	1	38.4695[a]	11,637.02[a]	0.0011	1.6720
Color × cream	1	0.0002	20.44	0.0012	0.4711
Color × copper	1	0.3724	129.26	0.0001	0.2667
Cream × copper	1	0.0528	191.85	0.0006	0.0819
Storage	2	11.3234[a]	5,413.94[a]	0.0962[b]	3.9104[a]
Storage × color	2	0.0515	36.01	0.0025	0.4763
Storage × cream	2	0.0754	126.33	0.0332	0.1522
Storage × copper	2	11.8140[a]	4,788.99[a]	0.0001	0.0882
Error	57	0.9634	54.90	0.0213	0.5623

[a] Significant at P = 0.01.
[b] Significant at P = 0.05.

tene accounted for 10% of the total vitamin A potency. Storage for 6 mo. at −5° F. did not affect the carotene content of the butter. Exposing the butter for an additional 2 wk. at 60° F., however, effected an 18% decrease, significant at P = 0.01, in the carotene content.

The preformed vitamin A content was not affected by the type of color, copper level, or storage.

The total vitamin A potency of the butter averaged 10,500 I.U. per lb. before storage. That storage effected a slight decrease, significant at P = 0.01, in the total vitamin A potency of the butter, is illustrated in Table 3.

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ESTROGEN CONTENT OF COLOSTRUM AND MILK OF DAIRY CATTLE [1, 2]

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SUMMARY

A study of the permeability of the secreting mammary gland epithelial cell to circulating blood estrogens is reported. Milks were dried and fed to ovariectomized mice for ten days. The increase in uterine weight of these mice was compared to controls and to those fed graded amounts of diethylstilbestrol added to dried whole milk, to estimate the estrogen in milk. Milks from 11 nonpregnant cows, producing from 26.9 to 66.8 lb. per day, were assayed. The average uterine weight of the group was 10.48 mg., and uterine weight/% of body weight was 0.05. The milk of nonpregnant cows either is free of estrogen or it contains amounts which can not be detected by the assay method. Milks from 32 cows, pregnant from 27 to 268 days, were assayed. The milk of ten cows, pregnant from 27 to 97 days, averaged mouse uterine weights of 11.35 mg., with an average uterine weight/% body weight of 0.06. Milk of 11 cows, from 104 to 193 days pregnant, averaged uterine weights of 13.71 mg., with an average uterine weight/% body weight of 0.07. Milk from 11 cows, from 200 to 268 days pregnant, averaged uterine weights of 16.45 mg., with an average uterine weight/% body weight of 0.09. These data indicate that the normal lactating cells are only slightly permeable to the normal circulating estrogen of the cow's blood, even in late pregnancy, when estrogen secretion is high and secretion of milk is low. Commercial market milk samples were obtained monthly for 18 mo. The average uterine weight of mice was 11.32 mg. and uterine weight/% body weight was 0.06. Samples of colostrum from six normal cows and from three cows experimentally induced to lactate showed low uterine weight increases. These observations are significant, because the secretion of estrogen in late pregnancy is at a maximum and the rate of colostrum (milk) secretion is minimal. Seven nonpregnant cows, producing from 5.4 to 26.8 lb. per day, were fed diethylstilbestrol at from 10 to 20 mg. per day per 1,000 lb. live weight, for 2 wk. or more. Nine samples from cows fed 10 mg/day showed average uterine weight of 12.07 mg. and an average uterine weight/% body weight of 0.07. These data indicate that the secreting mammary gland epithelial cell either is nonpermeable or is only slightly permeable, to circulating natural estrogenic hormones or to synthetic estrogens (diethylstilbestrol) when fed at levels of from 10 to 20 mg/day/1,000 lb. body weight.

The normal pathways of excretion of estrogen in cattle, via urine *(13)* and feces *(6)*, have received study during the past 25 yr. Recently, El-Attar and Turner *(3)* studied the combined estrogen excretion in the urine and feces simultaneously, during various stages of pregnancy in cattle.

The problem of the permeability of the secreting mammary gland epithelium to blood estrogens, i.e., the presence of estrogen in the milk of dairy cattle, has received relatively little study. There are three phases of the problem to consider, the estrogen in colostrum which is secreted, in part, in late pregnancy and stored in the udder until after parturition (the period of bagging up); the estrogen in normal milk during recurring estrous cycles, and the estrogen in the milk during pregnancy. This latter period is of greatest importance, because

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[3] With the technical assistance of Mary E. Powell.

it is a time when the milk is used for human consumption, whereas the colostrum is usually fed to the calf.

Dairy cattle usually are bred between the 60th and 120th days of lactation, so that they will calve at yearly intervals. The estrogen secretion of the pregnant dairy cow is at first low, but increases slowly during the first 160 to 170 days. A rapid increase in estrogen secretion then occurs until parturition *(3)*. During the latter part of pregnancy, when the estrogen secretion is relatively high, the secretion of milk is declining. If the mammary gland is permeable to estrogen, one should expect considerable concentration in such milk or in the colostrum.

The present paper will report the results of an extensive study concerned with the assay of colostrum, milk from nonpregnant cows, and milk from cows during various stages of pregnancy, for the presence of estrogen. These data will be compared with assays of milk from cows fed low levels of diethylstilbestrol.

Courrier *(1)* observed that if daily doses of from 80 to 100 rat units of estrogen were given to lactating guinea pigs, after three or four days the nurslings showed opening of the vagina and mammary gland development. Lacassagne and Nyka *(4)* reported that human colostrum in amounts of 1.5 ml., injected subcutaneously, caused vaginal cornification in spayed mice. Walker and Stanley *(17)* injected 0.2 and 1.0 mg. of diethylstilbestrol dipropionate daily in castrate and intact lactating rats. Premature opening of the vagina of the nurslings occurred in most of the treated litters, indicating the presence of estrogen in the mothers' milk. Recently, Pope and Roy *(11)*, using chemical extraction procedures, reported that the estrogenic activity of bovine colostrum was of the same order as that of human colostrum, and of bovine and human pregnancy blood, but was much lower than that in bile and urine during pregnancy. The estrogens occurred mainly as conjugates in the aqueous phase of colostrum and were partly ketonic. The conjugated estrogens of separated colostrum from one cow were equivalent to 5.2 μg. estradiol-17β per liter. Munch *(9)* reported the presence of estrogen in the milk of Spotted Mountain cattle. Variations were observed in the estrogen concentration during estrous cycle and pregnancy.

In England, the effects of implants of diethylstilbestrol and hexestrol upon the initiation of lactation have been studied at Cambridge University and at the National Institute for Research in Dairying. Samples of milk from heifers there were assayed for the presence of estrogen by Lawson *et al. (5)*. It was reported that the amount of estrogen present was too small for assay by direct feeding to rats. Milk samples were then extracted and assayed by subcutaneous injection or intravaginal application in ovariectomized rats. Milk from cows implanted with diethylstilbestrol showed amounts varying from 0.4 to 1.0 μg. per pint; whereas, with hexestrol the estrogen varied from 0.01 to 0.5 μg. per pint.

Chas. Pfizer and Company *(10)* assayed the milk of cows fed 10 mg. of diethylstilbestrol daily. The milk was spray-dried. Ovariectomized mice received 3 g. of the dried milk daily for ten days. It will be noted that the uterine weight of the mice fed milk from the diethylstilbestrol-fed cows was lower than the control cows' milk.

TABLE 1
Assay of milk from normal cows and cows fed diethylstilbestrol
(Pfizer, 1955)

Source of milk	Mice	Body weight	Uterine weight	Uterine weight / Body weight
	(No.)	*(g.)*	*(mg.)*	*(%)*
Control cows	10	25.40	19.16	0.08
Stilbestrol-fed cows	10	26.60	16.38	0.06

Voelker and Becker *(16)* implanted diethylstilbestrol pellets containing 15, 30, 240, and 1,500 mg. into lactating cows. The milk was homogenized and fed to immature normal mice as the liquid part of their diet for seven days. Uterine weight increase was used as an index of the presence of estrogen. At low levels the uterine response was variable or absent; whereas, significant amounts of estrogenic activity were observed in the milk from cows implanted with the higher levels of diethylstilbestrol.

METHOD OF BIOASSAY OF MILK

The method of the bioassay of milk was a modification of that employed in the bioassay of residual estrogens in the tissues of steers *(12)*. The mice used in the assay were ovariectomized and the uterus allowed to involute for a period of ten days. During this period, the animals were fed laboratory mouse feed. The problem of the best mode of feeding the milk was studied. Liquid milk from three cows was fed to groups of ten mice, being supplied in a water bottle and cup. The body growth of these mice was poor and several failed to survive the ten-day assay period. It was concluded that the mice did not consume sufficient liquid milk to meet their nutritional requirements.

It was then decided, in subsequent work, to dry the milk under infrared heat lamps. By frequent stirring, a crumbly, dried product was obtained without scorching. The samples were then stored in the deep freezer until assayed. To a few groups, 90% dried milk and 10% ground mouse feed were fed. In all other assays, the dried milk was fed ad libitum as the exclusive source of feed to groups of ten mice for a period of ten days. The mice were then sacrificed, body weight determined, and the uterus dissected clear of fat and connective tissue and weighed immediately upon a Roller-Smith balance. Care was taken in dissection to cut the uterus cleanly at the vaginal end of the cervix.

Sensitivity of the Assay Method. In a previous report *(12)*, the effect of the addition of graded amounts of diethylstilbestrol to beef rib-eye tissue upon the uterine weight response of mice was determined. In the present report, a similar study was made, in which graded amounts of diethylstilbestrol were added to dried milk. The dried milk consisted of commercial dried nonfat milk, 450 g., to which was added 150 g. melted butter, giving a total of 600 g. of whole dried milk. The diethylstilbestrol was dissolved in an olive-oil carrier at the level of 0.9 μg/ml. Three ml. of the oil was mixed with 600 g. of dried milk. Each gram of dried milk contained 0.045 μg. of diethylstilbestrol. Each succeeding milk sample was increased by 3 ml. of oil containing the estrogen (Table 2). One control group of

TABLE 2

Effect of graded doses of diethylstilbestrol added to dried whole milk (25% fat)
(Ovariectomized young mature mice)

Amount of diethylstilbestrol	Mice	Mean body weight	Mean uterine weight		Uterine weight / Body weight	
(μg/g milk)	*(No.)*	*(g.)*	*(mg.)*		*(%)*	
Control (mouse feed)	7	26.9	11.8		0.04	
Control (dried milk)	10	22.4	12.9	11.2	0.06	0.05
Control (dried milk)	10	21.4	9.5		0.05	
0.045	10	19.3	10.0		0.05	
0.090	10	20.1	10.8		0.06	
0.135	10	15.2	15.3		0.11	
0.180	8	18.1	26.2		0.15	
0.225	10	16.3	27.3		0.17	
0.270	10	18.5	30.6		0.17	
0.315	10	20.1	39.3		0.20	
0.360	10	21.4	50.5		0.25	
0.405	10	21.5	57.2		0.27	

mice was fed commercial mouse feed. The results on control groups fed the whole dried milk are presented. The first of these two assays was conducted several months earlier than the second, which was conducted simultaneously with the assay of the graded amounts of estrogen. It will be noted that as the amount of diethylstilbestrol added to the dried milk increased, the mean uterine weight and the percentage of body weight gradually increased. These data may be referred to in estimating the diethylstilbestrol-equivalent in the milk of dairy cattle.

It will be noted that the addition of 0.045 and 0.09 μg/g dried milk did not stimulate the average uterine weight of the mice above the range of control values, although the assay of the control sample, run simultaneously with the graded amounts of estrogen, was lower. These data are interpreted as indicating that the assay method can not detect amounts of estrogen in milk of 0.09 μg/g or less.

Assay of milk from nonpregnant cows. To determine whether assayable amounts of estrogen are present in the milk of nonpregnant cows, milk samples were collected, dried, and assayed. The group of 11 cows of the station herd was in various stages of lactation, but had not been rebred (Table 3). Whether any

TABLE 3

Assay of milk from nonpregnant cows

Cow No.	Breed	Milk yield	Assay data: Mice	Body wt.	Uterine wt.	Uterine wt. / Body wt.
		(lb/day)	*(No.)*	*(g.)*	*(mg.)*	*(%)*
400	Holstein	55.4	10	17.4	7.84	0.05
314	Holstein	41.5	10	19.8	11.41	0.06
397	Holstein	32.9	10	21.3	10.60	0.05
429	Holstein	49.3	10	19.3	11.78	0.06
9	Brown Swiss	26.9	10	21.8	9.68	0.05
433	Holstein	58.8	10	21.3	10.08	0.05
337	Holstein	57.7	10	19.1	9.36	0.05
587	Jersey	38.0	10	22.8	10.07	0.05
417	Holstein	49.8	10	19.2	12.00	0.06 (Pasture)
3	Brown Swiss	47.0	8	18.8	10.24	0.06 (Pasture)
376	Holstein	66.8	8	20.1	12.17	0.06 (Pasture)

of the cows were in estrus during the milk-collection periods was not determined. It will be noted that three cows were on pasture when samples were collected. Cows of the Brown Swiss, Holstein, and Jersey breeds, varying in production from 26.9 to 66.8 lb. per day, were included. Milk from two of the cows on pasture produced uterine weights of 12 mg. or more. The nine other cows ranged from a low of 7.84 to 11.78 mg. The average uterine weight of the group of 11 milk samples was 10.48 mg., and uterine weight/% of body weight was 0.05. Since these uterine weights are within the range of the control milk samples, it is concluded that the milk of nonpregnant cows either is free of estrogen or contains amounts of estrogen which can not be detected by the assay method employed. It is of interest that two cows on pasture produced milk showing the highest uterine weights. Whether plant estrogens may have contributed to their response is not known.

Assay of milk from pregnant cows. Milk from cows pregnant for various periods was collected, dried, and assayed as described. These cows were in various stages of lactation. The cows pregnant for the longer periods were usually

TABLE 4

Assay of milk from pregnant cows

Cow No.	Breed	Duration of pregnancy	Milk yield	Assay data			
				Mice	Body wt.	Uterine wt.	Uterine wt. / Body wt.
		(days)	*(lb/day)*	*(No.)*	*(g.)*	*(mg.)*	*(%)*
381	Holstein	27	16.9	10	21.6	10.56	0.05
611	Jersey	38	11.4	10	20.6	11.28	0.06
591	Jersey	45	13.7	10	18.6	11.12	0.06
443	Holstein	60	19.1	10	22.2	11.02	0.05
171	Holstein	70	35.0	10	23.8	11.10	0.05
600	Jersey	70	19.1	10	19.8	10.48	0.05
592	Jersey	77	24.0	10	19.2	11.12	0.06[a]
160	Jersey	82	17.9	10	20.4	14.73	0.07
420	Holstein	91	21.9	10	23.1	11.24	0.05
1	Brown Swiss	97	46.1	10	20.8	10.87	0.05
411	Holstein	104	26.8	9	16.9	8.73	0.05
590	Jersey	111	16.0	10	19.9	11.48	0.06
603	Jersey	152	24.6	10	20.5	11.44	0.06
370	Holstein	155	24.8	8	20.8	11.11	0.04
6	Brown Swiss	158	23.4	9	20.0	19.95	0.10
587	Jersey	160	9.3	11	20.8	13.70	0.07
436	Holstein	170	15.4	10	20.8	21.60	0.10
407	Holstein	178	21.9	10	19.1	11.71	0.06
8	Brown Swiss	181	19.8	10	19.3	17.81	0.09
598	Jersey	184	12.4	8	22.6	11.83	0.05
438	Holstein	193	12.4	10	17.9	11.48	0.06
434	Holstein	200	9.3	11	19.4	20.38	0.11
351	Holstein	206	23.4	7	18.4	11.14	0.04
7	Brown Swiss	207	14.3	9	20.1	19.05	0.10
417	Holstein	208	13.3	10	19.6	10.25	0.05
433	Holstein	210	15.5	9	18.0	16.56	0.09
346	Holstein	219	12.9	10	17.7	11.32	0.07
612	Jersey	222	7.2	9	18.1	43.53	0.24
304	Holstein	231	11.2	10	18.9	10.04	0.05
3	Brown Swiss	233	7.7	10	20.3	13.06	0.07
160	Jersey	238	7.1	8	17.3	13.38	0.08
160	Jersey	268	6.8	10	19.4	12.22	0.06

[a] Pasture eight days.

approaching the end of the lactation period and were producing only relatively small amounts of milk. The daily milk yield at the time the milk was collected is indicated in each case (Table 4).

The milk of ten cows from 27 to 97 days pregnant produced average mouse uterine weights of 11.35 mg., with an average uterine weight/% body weight of 0.06. Only a single cow in this group produced milk which stimulated uterine weight in excess of the control range.

The milk of 11 cows from 104 to 193 days pregnant produced average uterine weights of 13.71 mg., with an average uterine weight/% body weight of 0.068. In this group were three cows, each of whose milk was estimated to contain between 0.14 and 0.18 μg/g diethylstilbestrol-equivalent. The milk of the other cows was within the normal range.

The milk of 11 cows from 200 to 268 days pregnant produced average uterine weights of 16.45 mg., with an average uterine weight/% body weight of 0.09. Milk from one cow 222 days pregnant produced the greatest uterine response, 43.53 mg. The milk of three other cows in this group ranged from 16 to 20 mg., indicating a diethylstilbestrol response equivalent to about 0.18 μg/g dried milk. Four cows ranging from 222 to 268 days pregnant secreted milk producing uterine weights ranging from 10 to 13 mg.

In a concurrent study of the total estrogen excretion in the urine and feces of pregnant cows *(3)*, it was shown that low levels of estradiol-equivalent (4-5 mg/day) are produced during the first 160 days of pregnancy. Then there is a rapid increase in estrogen excretion to a level of about 20 mg/day, from 200 days to term. Although there is considerable variability in estrogen excretion in individual cows, the fourfold increase in estrogen during the latter part of pregnancy (200-280 days) might well have been expected to be reflected in an increased estrogen content of milk during this period. There was a slight average increase in uterine weight in this group, but it was owing, to a considerable extent, to a single milk sample.

These data on the estrogen content of the milk of pregnant cows are interpreted as indicating that the normal functioning mammary gland epithelial cells either are not permeable or are only slightly permeable to the normal circulating estrogens of the blood even in late pregnancy, when the estrogen secretion rate is high and the secretion of milk is low.

Assay of commercial market milk. To determine the amount of natural estrogens in commercial market milk, samples of processed milk from the University dairy sales room were obtained at monthly intervals, dried, and assayed. This milk was produced by the herds of the University at Columbia and represented milk from cows in all stages of lactation and pregnancy.

The first two samples obtained in August and September (1955), when the cows were on pasture, showed evidence of estrogen stimulation (Table 5). It was thought that the estrogen activity might have been due to the transfer of plant estrogens into the milk. The following spring, when the cows again were turned on pasture, a slight increase in estrogen activity in the milk again was observed one month, but was only slightly above the normal range. During the

TABLE 5
Assay of commercial milk for estrogen

Date of collection	Mice	Body wt.	Uterine weight	Uterine wt. / Body weight
1955	*(No.)*	*(g.)*	*(mg.)*	*(%)*
August 8	9	16.4	17.1	0.10
September 15	8	18.1	18.3	0.10
December 23	10	18.8	10.6	0.06
1956				
January 25	10	22.3	10.7	0.05
February 27	10	16.9	10.1	0.06
March 20	10	22.0	11.5	0.05
April 23	10	20.5	10.1	0.05
May 21	10	19.0	13.6	0.07[a]
June 18	10	18.4	10.9	0.06[b]
July 20	10	21.2	11.2	0.05
August 20	9	20.4	11.3	0.06
September 20	10	21.8	10.5	0.05
October 20	6	21.3	9.4	0.05
November 20	10	23.6	9.5	0.04
December 20	10	22.3	9.7	0.04
1957				
January 20	10	23.6	10.2	0.04
February 20	9	20.9	9.4	0.05
March 20	9	23.6	9.7	0.04

[a] Pasture 21 days.
[b] Pasture.

summer and fall, no further evidence of increased estrogenic activity was observed.

During the period of the 18 mo. that the samples of milk were assayed, the uterine response varied from 18.3 to 9.41 mg., with an average of 11.32 mg., and uterine weight/% of body weight of 0.06. These data lend further support to the thesis that estrogenic hormones are only slightly, if at all, permeable to the mammary gland epithelium.

Estrogen in colostrum and early lactation. Samples of colostrum and milk obtained at 10, 30, 60, and 90 days following parturition have been obtained from four individual cows and at ten to 30 days in two additional animals (Table 6). These data show that the colostrum (first milk) contains very little estrogen, the uterine weights ranging from 11.46 to 14.16 mg., with an average of 12.70 mg. and uterine weight/% of body weight of 0.06. This is very remarkable, because at this time the amount of circulating blood estrogen is at a peak (at time of parturition) and the functional activity of the mammary glands cells is low. Further, the fact that the cells at this time are especially permeable to blood globulins, which are in high concentration in colostrum, might lead one to expect high estrogen permeability at this time. Estrogens in circulating blood are believed to be combined loosely with blood protein (estroprotein), which might increase their permeability to the cell. Apparently, this binding is not sufficient to permit the passage of estroprotein into the colostrum in appreciable amounts.

With the advance in lactation, there appears to be a tendency for the uterine

TABLE 6

Estrogen content of colostrum and milk from early lactation

Cow No.	Breed	Remarks	Assay data			
			Mice	Body weight	Uterine weight	Uterine wt. / Body wt.
			(No.)	*(g.)*	*(mg.)*	*(%)*
402	Holstein	Colostrum	7	20.9	14.16	0.07
		10 days	9	23.0	12.80	0.05
		30 days	9	19.7	38.31	0.20
		60 days	9	17.7	10.14	0.06
		90 days	10	19.8	10.57	0.05
160	Jersey	Colostrum	10	19.6	13.85	0.07
		10 days	10	21.6	11.46	0.05
		30 days	10	19.4	9.19	0.05
		60 days	10	21.3	17.25	0.08
		90 days	10	21.0	8.53	0.04
583	Jersey	Colostrum	9	19.2	13.13	0.07
		10 days	10	24.7	10.17	0.04
		30 days	10	22.8	10.11	0.05
		60 days	10	19.1	9.83	0.05
		90 days	10	18.1	8.15	0.05
159	Jersey	Colostrum	10	22.0	11.77	0.06
		10 days	10	26.8	10.90	0.04
		30 days	9	20.5	8.74	0.04
		60 days	10	19.9	8.65	0.04
		90 days	10	22.0	8.91	0.04
585	Jersey	Colostrum	10	20.7	11.46	0.06
		10 days	10	20.3	9.13	0.05
		30 days	10	21.5	11.11	0.05
601	Jersey	Colostrum	10	21.8	11.85	0.06
		10 days	10	21.2	9.52	0.05
		Lactation experimentally induced				
594	Jersey	Colostrum	8	17.4	11.69	0.07
		10 days	9	19.4	13.59	0.07
		30 days	10	19.8	9.20	0.05
		60 days	10	19.5	9.40	0.05
		91 days	6	21.3	9.60	0.05
3625	Guernsey	Colostrum	10	19.5	9.88	0.05
18	Guernsey (Freemartin)	Colostrum	10	22.2	11.64	0.05

weights of the assay animals to decline slightly, although an increased uterine response was observed in milk samples from two animals at 30 and 60 days of lactation. No explanation can be offered for these higher responses.

It is believed that these observations are most critical in regard to the permeability of estrogen to the mammary gland epithelial cells, because the secretion of estrogen is at a maximum (as indicated by estimations of estrogen in the urine and feces in late pregnancy) and the rate of milk secretion (colostrum) in late pregnancy is minimal.

Effect of feeding diethylstilbestrol upon the estrogen content of milk. It was shown in our laboratory that the injection of estrogen into experimental animals stimulated an increased secretion of the lactogenic hormone. It has been suggested that the initiation of lactation in normal pregnant animals at the approach of parturition is owing to an increased secretion of estrogen *(7, 8)* which, in turn,

stimulates the increased secretion of the lactogenic hormone. Based on this theory, estrogen was administered to the experimental sterile heifers after udder growth had been produced. That estrogen is effective in initiating lactation at this time has been demonstrated *(14)*.

In the sterile heifers induced to lactate by this method, there was a gradual increase in milk yield, reaching a peak, then a gradual decline, similar to the decline observed in normal lactating cows. In a group of these heifers, the feeding of 10 mg. of diethylstilbestrol daily tended to prevent the decline in lactation observed. It was suggested that the feeding of estrogen might be an effective method of maintaining a higher level of secretion of the lactogenic hormone. The data presented *(14)* indicated such a role.

The problem arose as to whether the feeding of diethylstilbestrol or other synthetic estrogens to cows, after lactation started to decline, would increase the estrogen content of the milk. Whereas the previous results indicated that the natural estrogens are either nonpermeable or only slightly permeable to the lactating epithelial cell, within the normal range of circulating estrogens during pregnancy, there might be a difference in the permeability of the gland cells to the synthetic estrogens such as diethylstilbestrol.

A group of nonpregnant cows, ranging in production from 5.4 to 26.8 lb. per day, was fed diethylstilbestrol at levels varying from 10 to 20 mg. per day for periods of 2 wk. or more. Lower-producing cows were used, in order to accentuate the concentration of the estrogen if the cells showed indications of permeability to the hormone. The nine milk samples from cows fed 10 mg/day showed a range in uterine weight from 17.56 to 7.67 mg., with an average of 12.07 mg., and an average uterine weight/% body weight of 0.07. The two samples from cows fed 15 mg/day averaged 9.65 mg., whereas one sample from a cow fed 20 mg/day produced an average uterine weight of 15.44 mg. (Table 7).

These milk samples, considered as a group, show minimal stimulation of the

TABLE 7

Diethylstilbestrol feeding on estrogen content of milk

Cow No.	Breed	Milk yield	Remarks	Assay data			
				Mice	Body weight	Uterine weight	Uterine wt. / Body wt.
		(lb/day)	*(mg.)*	*(No.)*	*(g.)*	*(mg.)*	*(%)*
310	Jersey	15.0	10[a]	10	15.1	11.04	0.07
580	Jersey	22.1	10[a]	10	15.9	13.98	0.09
		15.4	10	10	19.8	10.30	0.06
585	Jersey	12.6	10	10	21.8	11.68	0.05
		26.8	15	10	23.9	10.00	0.04
544	Jersey	13.5	10	9	13.0	17.08	0.14
		17.2	10	10	17.9	17.56	0.10
		18.1	20	10	24.1	15.44	0.07
		12.8	10	10	20.4	9.36	0.05
594	Jersey	9.8	10	9	18.2	7.67	0.04
601	Jersey	28.4	10	10	23.1	9.94	0.04
3596	Guernsey	5.4	15	10	19.4	9.30	0.05

[a] 90% Dried milk + 10% mouse feed.

uterus. The samples showing the greatest estrogenic activity indicate concentrations of diethylstilbestrol equivalent to 0.14 μg/g of dried milk, which is comparable to some samples of milk from cows in late pregnancy.

From these data, it is concluded that the feeding of diethylstilbestrol at levels of from 10 to 20 mg/day to lactating cows permits the passage into the milk of either no estrogenic hormone or minimal amounts of it.

Estrogen assay of milk from cows experimentally induced to lactate. A number of sterile heifers have been treated with estrogen and progesterone for a period of 180 days, to stimulate the growth of the udder. At the end of this time, the treatment was stopped and lactation was initiated by the daily injection of 3 mg. of estradiol benzoate, for a period of 14 days. Milking was started at this time. The yield of milk was limited for a few days, then increased rapidly for a period of weeks *(14)*. To determine the amount of estrogen present in this first milk (colostrum?), samples from a Jersey and two Guernsey cows were dried and assayed. In the two Guernsey cows, only the colostrum was assayed; whereas, in the Jersey (No. 594) samples were obtained at intervals up to 90 days, as in the previous study. It will be noted that only minimal stimulation of the uterus was induced, comparable to the observations in the cows which calved normally (Table 6).

DISCUSSION

The present study was initiated to determine the extent to which the functional mammary gland serves as a normal pathway of excretion of estrogen in dairy cattle. In the human, as well as in cattle and some other domestic animals, lactation and pregnancy are concurrent. During pregnancy, the placental secretion of estrogen gradually increases, reaching a peak in late pregnancy. If the epithelial cells of the lactating mammary gland were permeable to estrogen, one might expect the presence of increasing amounts of estrogen in the milk secreted in late pregnancy and in the colostrum after parturition.

The observations pesented indicate clearly that such is not the case. Within the sensitivity of the assay method, milk is essentially free of estrogens even during periods of highest estrogen secretion and low milk secretion. The data showing the extremely low levels of estrogenic activity in colostrum, which is secreted at a time when the mammary cells are permeable to blood globulins and certain other blood constituents, are of special significance.

The discovery of the presence of isoflavones with estrogenic activity in clovers and soybean meal has raised the question of the possible presence of these compounds in milk. Although a slight increase in estrogenic activity was observed in some samples of milk from cows on pasture, significant levels of estrogenic activity which could be ascribed to plant sources are not believed to be present in milk.

The feeding of synthetic estrogens such as diethylstilbestrol to lactating dairy cattle, to stimulate the continued secretion of high levels of the lactogenic hormone and thus maintain intense milk secretion during the normally declining period of lactation, is believed to hold promise. The question of the permeability of the mammary gland epithelium to the synthetic estrogens is naturally raised.

The present study indicates that the levels of diethylstilbestrol, i.e., from 10 to 20 mg. per day to cows weighing about 1,000 lb., is not sufficient to permit the passage of significant amounts of estrogen into the milk. If estrogen is present in the milk, it is at the border line of sensitivity of the assay method employed. As a means of comparison, it should be pointed out that the samples showing the greatest estrogenic activity were estimated to show the diethylstilbestrol-equivalent of 0.14 μg/g dried milk. It has been shown that to stimulate the growth of the mammary gland duct system of male mice in a period of from 3 to 4 wk., it would require 1.23 μg/g of mouse feed *(2)*, or about ten times the amount estimated to be present in the milk.

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THYROIDAL UPTAKE OF I^{131} BY DAIRY CALVES [1]

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SUMMARY

This study shows some of the factors causing variation in the 48-hr. uptake of radioactive iodine in 83 dairy calves for 2 yr. The average uptake was 36.4% of the injected dose, with considerable variation within and between trials. The Brown Swiss calves averaged 10% lower than calves of the other breeds. The highest average per cent of uptake was found in the summer, and the lowest in the spring. Hourly counts calculated as the per cent of the 24-hr. count showed little difference in the rate of uptake between breeds. A statistical analysis of the uptake of 64 Brown Swiss, Holstein, and Jersey calves showed a significant difference due to breed, season, breed × season, and other interactions. The age differences were not significant. A correlation coefficient of −0.17 was found when 37 observations of the 48-hr. uptake and the estimated thyroxine secretion rate were analyzed. The addition of iodized salt to the ration, and injecting the isotope intravenously rather than subcutaneously, had no apparent effect on the percentage of uptake. From the findings in this study and those of other workers, it appears questionable whether the thyroidal uptake of I^{131} alone will provide worthwhile information on the thyroid status of dairy calves.

One of the methods used to measure changes in the activity of the thyroid is to determine the per cent of an injected dose of I^{131} collected by the thyroid over a period of time after injection of the isotope. Werner *et al. (9)* and others used the 24-hr. uptake of radioactive iodine as the most stable time at which to assay thyroid function in humans. The uptake of radioactive iodine has been used by Swanson *et al. (7, 8)* and by Blincoe and Brody *(2, 3)* as the basis for determining the effect of several factors on thyroid activity in dairy cows. However, it has not been demonstrated in the dairy animal that the uptake of radioactive iodine is a reliable measure of thyroid activity. This paper describes some of the factors which may affect the uptake of radioactive iodine by the thyroid gland of dairy calves, and also correlates some of these values with actual thyroid secretion rates.

EXPERIMENTAL PROCEDURE

The experimental animals were from the Michigan State University dairy herd and included calves of the Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey breeds. The average age was 5.2 mo., with a standard deviation of 2.2 mo. The calves received the regular rations used at the University which, in the early trials, did not include supplemental iodine. However, for the later trials the ration was supplemented with iodine by mixing 1% of iodized salt containing 0.02% potassium iodide into the concentrate.

A tracer dose of carrier-free I^{131}, ranging from 50 to 150 μc., was injected either subcutaneously or intravenously into each heifer. The uptake of I^{131} by

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the thyroid gland was followed by taking counts of the radioactivity from the thyroid area of the neck at hourly, daily, or alternate-day intervals. As far as possible, the same amount of pressure was applied to the neck of the calf each time counts were taken. The percentage of uptake was calculated from the maximum repeatable count obtained from this region of the neck. Corrections were made for background and the physical decay of the isotope. A Co^{60} source was used to standardize the instruments.

During the earlier studies, a shielded end-window TGC-8 Geiger-Mueller Tube (Tracelab) was used with a Model 1615 B count-rate meter (Nuclear). A scintillation counter and Model 1620 count-rate meter (Nuclear) were used for the later work, allowing a reduction in the amount of isotope injected into each animal because of the greater sensitivity of the instruments.

Aliquots of the injection solution were counted in a "phantom neck," similar to that described by Blincoe *(1)* for use as a standard in determining the percentage of uptake.

A series of trials was conducted during a period of 2 yr., with eight calves being used in each trial.

RESULTS AND DISCUSSION

Usually, the highest degree of thyroid radioactivity was reached about 48 hr. after injection. However, some animals showed a maximum count on the first day and many animals continued to accumulate I^{131} for a week or more.

Considerable variation in the 48-hr. uptake of I^{131} was found within, as well as between, trials. Some of the factors causing these differences could have been age, breed, season, level of iodine in the ration, and route of administration of the isotope. From this study, the major factors involved appear to be breed and season. The average 48-hr. uptake of 83 calves was 36.4% of the injected dose. The average percentage of uptake according to breed is shown (Table 1). The largest difference was found between the Brown Swiss and the other breeds. There was considerable variation in uptake between individuals within a breed, but the Brown Swiss calves averaged about 10% lower in comparison with the average uptake of calves from the other breeds.

A large amount of variation in the 48-hr. uptake was noted during the course of the study for different months during the year. In order to make a comparison, the year was divided into the four seasons: Winter—January, February,

TABLE 1

Effect of breed on the 48-hr. uptake of I^{131} by the thyroid gland of dairy calves

Breed	No. of calves	Percentage uptake	Standard deviation
Ayrshire	3	44.7	.....
Brown Swiss	26	29.3	13.8
Guernsey	8	38.9	15.7
Holstein	22	37.9	13.6
Jersey	24	40.9	17.4
Average	83	36.4	

March; spring—April, May, June; summer—July, August, September, and fall—October, November, and December. The average uptake for the four seasons is shown (Table 2). The highest uptake occurred in the summer and the lowest in the spring. These seasonal differences in uptake are not surprising, in view of the observation of Seiden and Fenger *(6)* that the iodine content of cattle thyroids was highest in late summer and at a minimal level in the late winter. However, it is well known that in other species of animals thyroid secretion is lowest in the summer and highest in late winter. Although there are few data to show any seasonal differences in the thyroid secretion rate of dairy animals, there is no reason to believe it would be the reverse of other species. Therefore, it is believed that the iodine content of the thyroid gland is not indicative of the gland's activity but only shows its storage capacity. The same can be said for uptake of radioactive iodine, in that it is a measure of the collecting ability of the gland and, owing to storage of iodine, may not be indicative of the output of the gland. Based on the results of this study, the 48-hr. uptake indicates a period of high storage of radioactive iodine in the summer and a period of low storage in the spring.

So few Ayrshires and Guernseys were studied that an adequate comparison of all breeds could not be made. However, a statistical analysis was made to determine the age, season, and breed differences in the percentage of uptake of I^{131} of 64 Brown Swiss, Holstein, and Jersey calves. Because of the distribution of the calves in each classification, a method of least squares was used to determine the effect of each component. Distribution of the animals according to breed and season, and the variation in the 48-hr. uptake, are shown (Table 3). The ages of the 64 calves included in the analysis ranged from 3 to 9 mo. To determine the variation due to season, the year was divided into the four seasons, as previously described.

TABLE 2

Effect of season on the 48-hr. uptake of I^{131} by the thyroid gland of dairy calves

Season	No. of calves	Percentage uptake	Standard deviation
Winter—(January–March)	24	38.4	9.7
Spring—(April–June)	24	26.7	10.4
Summer—(July–September)	12	53.6	18.5
Fall—(October–December)	23	35.5	15.5

TABLE 3

Breed and seasonal variation in 48-hr. uptake of I^{131}

Breed	Winter		Spring		Summer		Fall	
	No.	Uptake	No.	Uptake	No.	Uptake	No.	Uptake
		(%)		*(%)*		*(%)*		*(%)*
Brown Swiss	4	31.2	9	20.5	5	48.5	5	26.0
Jersey	7	40.6	8	23.6	3	70.3	7	44.6
Holstein	5	40.8	4	40.6	4	47.2	3	24.8

The component effects of age, breed, and season, as determined by method of least squares, are shown (Table 4) by the deviation from the over-all average; the analysis of variance also is shown (Table 5).

Results of the analysis show a significant difference due to breed, season, breed × season interaction, and the other interactions. The age differences were not significant, which is what one might expect since the range in ages was not large. However, there is an indication that the younger calves had a higher average uptake than did the older calves. The most important finding from the analysis was the highly significant breed × season interaction. This interaction, along with the breed or season differences, further complicates the use of the uptake of radioactive iodine in making comparisons of dairy calves because, when making a breed or season comparison, one has to specify the particular breed and season being compared. The other interactions could not be divided into individual component parts because of the distribution of the animals, but their significance is probably due to the breed and season differences.

TABLE 4

Component effects shown by deviation from the average 48-hr. uptake of I^{131}

General effects	Deviation from av.[a]	Interactions	Deviation from av.[a]
Age (mo.)			
3	7	Winter ×	
4	6	Brown Swiss	− 2
5	10	Jersey	2
6	− 4	Holstein	0
7	− 4		
8	− 5	Spring ×	
9	− 9	Brown Swiss	1
		Jersey	−11
Breed		Holstein	10
Brown Swiss	− 9	Summer ×	
Jersey	8	Brown Swiss	1
Holstein	0	Jersey	7
		Holstein	− 8
Season			
Winter	0	Fall ×	
Spring	−12	Brown Swiss	0
Summer	13	Jersey	2
Fall	− 1	Holstein	− 2

[a] Average = 35.

TABLE 5

Analysis of variance

Source	d.f.	s.s.	m.s.
Age	6	1,171	195
Breed	2	2,426	1,213[a]
Season	3	4,265	1,422[a]
Breed × season	6	1,652	275[b]
Other interactions	24	3,590	150[a]
Within cells	22	1,601	73

[a] Significant at 5%.
[b] Significant at 1%.

Since Michigan lies in a goitrous area, and the calf rations in the earlier trials did not include supplemental iodine, it was thought that the addition of supplemental iodine to the ration might have an effect on the uptake of I^{131}. About midway in the study, iodized salt containing 0.02% potassium iodide was mixed in the concentrate ration at the level of 1%. The addition of the supplemental iodine, at the level added, had no apparent effect on the uptake of I^{131} by the thyroid gland of calves, when compared with trials conducted in the same season the previous year, when no supplemental iodine was included. However, the amount of iodine administered might not have been in excess of the animals' needs, and further study needs to be done to determine the effect of an excess of iodine on the uptake.

It was also thought that intravenous injection of the isotope might possibly have an effect on the 48-hr. uptake, compared to subcutaneous injection. To determine this, the calves in three trials were injected intravenously in the jugular vein. No difference was found in the percentage of the injected I^{131} collected by the thyroid gland in 48 hr. by this route of injection, when compared with the uptake by calves injected subcutaneously.

Hourly counts for a period of 8 hr. were obtained from the calves receiving the intravenous injections, to show the rate of uptake of the isotope. The rate of uptake was calculated as the per cent of the 24- or 48-hr. count. The per cent of 24-hr. count for the first 8 hr. of 24 calves is shown (Table 6). When the per cent of the 24- or 48-hr. count is plotted, the accumulation of I^{131} for the total period of time is curvilinear, but it may approach linearity over a small segment of time, particularly during the first few hours after injection. Even though there was a difference between breeds, in the per cent of the injected dose collected up to 48 hr., when calculated in the manner above, there does not appear to be a large difference in the rate of uptake. The percentage increase for each hour, except for the first hour, shows that the thyroid gland of the Brown Swiss calves collected I^{131} at about the same rate as did the calves of the other breeds.

TABLE 6

Hourly uptake of I^{131} expressed as percentage of 24 hr.

Breed	No. of calves	Percentage by hour						
		1	2	3	5	6	7	8
Holstein	10	11.4	17.7	22.4	33.3	36.8	40.0	46.5
Brown Swiss	8	6.1	11.0	15.8	25.4	29.4	30.9	38.4
Jersey	4	8.3	15.1	19.8	29.4	35.0	41.0	44.3
Guernsey	2	10.2	16.0	22.7	34.4	38.3	41.8	.....
Average		9.0	14.9	19.8	30.1	33.9	37.5	43.4

The present authors have reported a technique for estimating the daily thyroxine secretion rate of dairy calves *(5)*. It is the best method available at the present time for determining the actual output of the thyroid hormone which, without question, is the best criterion for measuring gland activity. A correlation analysis was run on 37 observations of the 48-hr. uptake of I^{131} and the

estimated thyroxine secretion rate. The correlation coefficient of −0.17 was not statistically significant. However, there appears to be a tendency toward an inverse relationship between the 48-hr. uptake and the thyroxine secretion rate, as found in this study. This same relationship has been reported for mice by Chai *et al. (4)*. In the light of this study, as well as the findings cited earlier, it appears extremely questionable whether thyroidal I^{131} uptake alone will provide worthwhile information on the thyroid status of dairy calves.

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EFFECT OF HAY-CONCENTRATE RATIO ON RUMEN FILL AS MEASURED BY AN ANTIPYRINE DILUTION TECHNIQUE [1]

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SUMMARY

The rumen contents of seven fistulated bovines were removed and weighed at from 2 to 24 hr. after feeding. These 477- to 1,310-lb. animals were fed from 1.3 to 3.1 lb. of air-dry material per 100 lb. body weight per day in one portion. Regression equations were developed for the total contents and water contained in the reticulo-rumen as a function of the per cent concentrate in the ration, time after feeding, and plane of nutrition. The per cent concentrate in the ration had a much larger effect on the amount of rumen contents than either time after feeding or plane of nutrition. Hay-concentrate ratios did not significantly affect dry matter concentrations in the rumen. The dry matter content of rumen ingesta decreased with time after feeding ($P < 0.01$). An antipyrine dilution method was developed for estimation of the water content of the reticulo-rumen. The regression equations were found to give a better estimate than the antipyrine dilution values.

The proportion of the body weight which is constituted by the contents of the forestomachs has been of interest in research on body composition *(7)*, appetite *(6)*, and rumen fermentation *(3, 4)*. The mass of the reticulo-rumen contents is of direct economic importance as a factor in the shrinkage or fill involved in marketing cattle *(1)*. Recent reviews indicate that considerable variability exists in the mass of the reticulo-rumen contents, but they give little information concerning the cause *(3, 6)*. The present study was undertaken to study these factors and to develop a simple method for measuring the mass contained in the reticulo-rumen of living cattle.

METHODS AND PROCEDURES

The four cows, one heifer, and three steers used in this study were equipped with the plastic fistula plug of Hentschl *et al. (5)*. The cows were dry, but represented all stages of pregnancy. All animals were fed from 1.3 to 3.1 lb. of air-dry material per 100 lb. of body weight once daily at 8 A.M. Body weights ranged from 477 to 1,310 lb. The contents of the reticulo-rumen were removed, weighed, and sampled at various times between 2 and 24 hr. after feeding. Measurements were made after a 3-wk. stabilization period on each ration. The rations contained from 0 to 80% concentrate. The concentrate varied slightly, but was based on a mixture of four parts ground corn to one part of soybean oil meal. The remainder of the ration was alfalfa or alfalfa-grass hay which varied greatly in quality and stage of maturity.

The water content of the rumen was also determined by mixing with the fist from 10 to 50 ml. of 10% antipyrine solution throughout the rumen contents for

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a period of 2 min. Mixing was repeated after a 5-min. interval, and a sample withdrawn. This sample was then centrifuged at 14,000 r.p.m. for 20 min. and the antipyrine concentration in the supernatant determined by the chloroform extraction procedure of Brodie *et al. (2)*.

RESULTS AND DISCUSSION

The total contents and the water of the reticulo-rumen, as determined by removal of these contents, have been fitted to a multiple regression equation as described by Snedecor *(8)*. A total of 42 determinations are included:

Y = Total reticulo-rumen contents expressed as per cent of body weight

W = Amount of water in the reticulo-rumen expressed as per cent of body weight

X_1 = Per cent concentrate in the ration

X_2 = Time after feeding, in hours

X_3 = Plane of nutrition expressed as lb. of air-dry feed consumed per 100 lb. body weight.

$$Y = 12.21 - 0.05\ X_1 - 0.13\ X_2 + 2.65\ X_3$$
$$W = 11.85 - 0.05\ X_1 - 0.10\ X_2 + 1.78\ X_3$$

The standard errors of estimate for Y and W, respectively, are 2.72 and 2.82. Expression of Y and W as per cent of body weight implies an assumption of a linear relationship between these variables. The small standard errors of Y and W found in this study, involving a wide range of body weights, lend support to this assumption. Standard errors for the regression coefficients for X_1, X_2, and X_3, respectively, are about 0.01, 0.03, and 0.7. These relationships also are shown (Figure 1).

The regression coefficients for X_2, time after feeding, show that the total ingesta decreases faster than the water in the reticulo-rumen ($P < 0.01$). Thus, the dry matter content of rumen ingesta decreases with time after feeding when the amount and quality of the ration are constant. This is in agreement with Mäkalä *(6)*. A third regression equation for the dry matter of the rumen can be obtained by substracting W from Y. Dry matter concentration in the reticulo-rumen was not significantly affected by the hay: concentrate ratio, but did tend to increase with increasing plane of nutrition.

It has been assumed that Y and W vary in a linear manner with each of the X's. This is true for X_1 in this study, but the data are not adequate to test linearity with X_2 and X_3.

Highly significant ($P < 0.01$) multiple correlation coefficients of 0.70 and 0.64 were found for Y and W. It should be noted that around 30% of the variability remains to be accounted for. Factors which remain to be considered are quality of hay, stage of pregnancy, short- and long-term previous environment, degree of fatness, and inheritance. Quality of the forage probably is particularly important. Balch and Line *(1)* have observed 20 to 30% decreases in the mass of

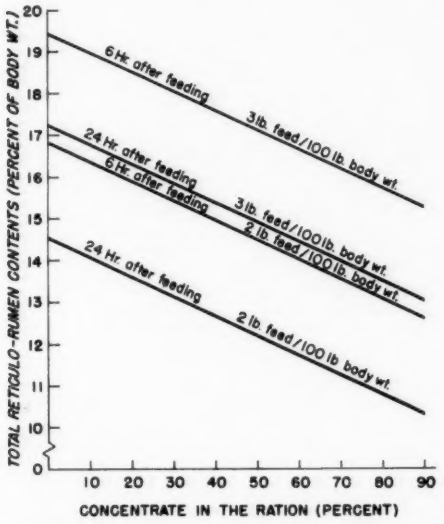

FIG. 1. Rumen fill as calculated from the regression equation.

the reticulo-rumen contents with a change from dry-feeding to pasture. Also, Stalleup *et al. (9)* reported that retention time of hays in the reticulo-rumen increases with increasing lignin content. Observations should be made on green forage and silage, also.

The standard partial regression coefficients (Table 1) are independent of units; thus, they express the relative contribution of X_1, X_2, and X_3 to Y and W. When each standard partial regression coefficient was divided by the sum of the 3, it was found that ration accounted for 44% of the variation considered in this study, whereas time after feeding accounted for 30%, and plane of nutrition, 26%.

TABLE 1

Standard partial regression coefficients and correlation coefficients

	X_1	X_2	X_3
Correlation between Y and X's	−0.55	−0.35	0.53
Correlation between W and X's	−0.55	−0.28	0.45
Standard regression coefficients of Y on X's	−0.41	−0.33	0.30
Standard regression coefficients of W on X's	−0.45	−0.27	0.21

The water content of the reticulo-rumen, as determined by the antipyrine dilution method just described, yielded values $131.6 \pm 7.9\%$ of the values determined by emptying the rumen. When the antipyrine was allowed to mix with the rumen contents naturally, distribution remained uneven for as long as 1 hr. Disappearance of antipyrine from the reticulo-rumen was uneven and unpredictable. The regression equations will give a better estimate than the antipyrine dilution value. The N-acetyl-4-aminoantipyrine method of Reid *et al. (7)* should be more suitable.

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COMPARISON OF IN VIVO AND IN VITRO TECHNIQUES IN RUMINOLOGY STUDIES [1]

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SUMMARY

This paper is concerned with the comparison of in vivo and in vitro rumen bacterial dissimilations of some carbohydrates, amino acids, and organic acids of metabolic importance. It was observed that succinate, DL-aspartate, glucose, and maltose were readily fermented by rumen microorganisms, and that volatile fatty acids (VFA) were produced. The amino acids DL-lysine, DL-alanine, and glycine were not degraded to an appreciable extent to VFA by rumen bacteria. In general, qualitative agreement between in vivo and in vitro washed cell suspension (WCS) experiments was obtained with DL-lysine, DL-alanine, and glycine, while the in vivo studies using DL-aspartate and succinate agreed with the WCS in vitro studies of Sirotnak *et al. (17, 18)*. With the substrates glucose and maltose, in vitro WCS experiments were both variable and in disagreement with the in vivo studies. Thus, the WCS technique appears most useful for studying short one- or two-step reactions presumed to occur in the rumen. This technique loses significance when studying multistep metabolic reactions and, in such cases, the results should be interpreted with caution until they are verified by in vivo experiments, or until further studies concerning the limitations of the WCS technique are available.

In vitro investigations of the rumen flora require the use of one, or a combination, of the following methods: Microscopic examinations, pure culture studies of bacteria, studies on cell-free enzymes from bacterial or protozoan suspensions, and various types of the so-called artificial rumen *(3)*. Because the determination of volatile fatty acids (VFA) in the rumen is, at best, only a rough estimate of production and can not be used for precise studies on the metabolism of rumen bacteria, Johns *et al. (9)*, Sijpesteijn and Elsden *(16)*, and later, Doetsch *et al. (5)* have used buffered washed cell suspensions (WCS) of rumen bacteria in an attempt to add percision to such studies. Elsden *(16)* stated the following advantages for this technique: *(a)* The population is studied as an entity under controlled conditions; *(b)* the enzymatic action of the population in the absence of an added substrate is negligible; *(c)* since the experimental period is short and growth is not permitted, the chance of significant alteration in the composition of the population is nil, and *(d)* small-scale work is possible which permits the investigation of the degradation of expensive or toxic materials. This technique and its modifications have been described by Doetsch *et al. (5)*.

An extensive study was conducted by Doetsch *et al. (4)*, in which the washed cell suspension technique was used to study catabolic reactions of rumen bacteria. The dissimilation of numerous substrates was studied in regard to gas and VFA production. Similar studies on amino acids, polysaccharide synthesis, and lactic

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acid formation were reported by Sirotnak *et al. (17)*, Gibbons *et al. (8)*, and Jurtshuk and Hueter *(10)*. A later study was conducted by Sirotnak *et al. (18)* on aspartate dissimilation reactions of rumen bacteria. The results confirmed previous findings and, in addition, indicated the probable pathways of dissimilation of aspartic acid.

The data reported in this paper are concerned with the comparison of in vivo and in vitro rumen bacterial dissimilations of some carbohydrates, amino acids, and organic acids of metabolic importance. The purpose was to determine the extent to which in vitro dissimilations are representative of in vivo occurrences. Our intention was to make a simultaneous and more direct comparison between in vivo and in vitro results than has been done heretofore.

EXPERIMENTAL PROCEDURE

Animal feeding. Two permanently fistulated, nonlactating and nonpregnant cows (Jersey and Holstein) were used as experimental animals. They were fed slightly in excess of Morrison's recommended standards *(14)* on a ration of 8 lb. of alfalfa hay and 6 lb. of 16% protein concentrate mixture. The concentrate ration was fed twice daily except on days of experimental trials, when the morning feeding was omitted. Hay was fed only in the evening, and in controlled amounts to insure complete consumption by 12 midnight preceding a trial. On trial days, water was withheld from the animals starting at 12 midnight and ending at 4:30 A.M., at which time they were allowed access to water for approximately 0.5 hr. The source of water was then removed, and to afford sufficient time for the ingested water to reach equilibrium within the animal, an interval of approximately 3.5 hr. was allowed before the start of the trial. After the morning watering, the animals did not receive water again until the end of the trial period. The cows remained at approximately constant weight and were in good health throughout the entire study.

Sampling. Rumen liquor samples (500 ml.) were obtained via the fistula with the aid of a metal cannula and rubber hose connected to an aspirator pump. Samples were taken prior to administration of substrate and at intervals after substrate addition. Immediately after removal, the rumen liquor was strained through two layers of cheesecloth, then centrifuged at $1{,}000 \times G$ for 15 min. to remove the residual feed particles. Aliquot samples of the resultant supernatant liquid were preserved for lactic acid and volatile fatty acid analyses. Samples for lactic acid determination were preserved by adding 0.1 ml. of 100% (w/v) trichloroacetic acid to 1 ml. of rumen liquor. Samples for VFA determinations were preserved by adding 1 ml. of a saturated mecuric chloride solution to 9 ml. of rumen liquor.

The pH of rumen samples, when determined, was measured within 2 to 3 min. after removal. Although these measurements probably did not represent the precise intraruminal pH, it appears reasonable to assume that any large changes in rumen liquor pH would be detected by this procedure.

For in vitro dissimilation studies, cell suspensions were prepared as described by Doetsch *et al. (5)*. These cell suspensions were made from the rumen liquor

of an experimental animal, on which an in vivo study of the same substrate was being made simultaneously. Two different cell suspension dissimilations were performed for each in vitro study. One cell suspension, referred to hereafter as the 0-hr. suspension, was prepared immediately before adding the substrate to the rumen. The other cell suspension, referred to hereafter as the 5-hr. suspension, was prepared from the rumen liquor of the same cow 5 hr. after the administration to the rumen of the substrate being studied. The bacteria in the 0-hr. suspension had no previous contact with the substrate, whereas those of the 5-hr. suspension were exposed for 5 hr. to the particular substrate being investigated. The fermentation liquor of in vitro dissimilation studies was saved for VFA determinations. The samples were preserved as just described.

Rumen liquor lactic acid was determined by the method of Barker and Summerson *(1)* and the VFA by the method of Keeney *(11)*. The Beckman pH Meter, Model H-2, using standard glass electrodes, was used for measuring the pH of rumen liquor.

All substrates used in the in vivo studies were diluted with water to a volume of 1.5 to 2.0 liters, then administered via the rumen fistula. The following substrates were used for in vivo studies: sodium succinate, DL-aspartic acid, DL-aspartic acid neutralized to pH 6.9 with Na_2CO_3, D-glucose, maltose, DL-lysine, and a mixture of glycine plus DL-alanine. In order to compare in vivo with in vitro results, in vitro dissimilations were made on the same substrates, with the exception of succinate and aspartate. The dissimilation of these latter substrates by WCS has been studied in detail by Sirotnak *et al. (17, 18)*; hence, was not included.

RESULTS

Sodium succinate and DL-*aspartic acid.* Results of in vivo studies with sodium succinate and DL-aspartic acid are summarized (Table 1). As a control, the rumen liquor analyses for a cow from which feed had been withheld for 18 hr. preceding the sampling period are shown. It will be noted that the concentration of propionic acid in the rumen increased approximately 17 and 9 molar per cent, respectively, following additions of sodium succinate and neutralized DL-aspartic acid. Decreases occurred in the rumen liquor concentrations of acetic acid and butyric plus higher acid fractions following the administration of sodium succinate. A decrease also occurred in the acetic acid concentration of the rumen liquor of the cow which received neutralized DL-aspartic acid, with a concurrent decrease in butyric acid that appeared almost equal to the increases in valeric and higher acids. This increase in the valeric and higher acid fraction may have been owing to an increase in branched-chain fatty acids resulting from the Stickland-type reaction, as suggested by El-Shazly *(7)*.

The DL-aspartic acid which was not neutralized was not dissimilated to VFA. Indeed, the concentration of total VFA decreased more rapidly than in the fasting cow, probably owing to the low pH of the rumen liquor. No consistent or marked changes occurred in the relative concentrations of the various fatty

TABLE 1

Effect of in vivo dissimilation of sodium succinate and DL*-aspartic acid on rumen liquor composition and pH*

Trial No.	Treatment	Sampling time	Total VFA	Acetic	Propionic	Butyric	Valeric plus higher acids	Rumen liquor
		(hr.)	*(μM/ml)*	*(Molar % total VFA)*				*(pH)*
1	Previously fasted 18 hr.	0	91.4	68.7	16.1	13.2	1.9	6.9
		0.5	87.8	68.7	16.0	13.4	1.8	7.0
		2	78.2	68.5	16.1	13.3	2.1	6.9
		5	75.2	68.0	15.9	13.3	2.8	6.8
		8	54.8	68.5	15.3	13.6	2.6	7.0
2	1 kg. sodium succinate	0	21.3	69.5	18.9	11.6[a]	....	....
		0.5	49.6	66.5	19.7	13.8	....	....
		1.5	55.1	65.3	22.3	12.4	....	....
		3.5	56.4	61.5	27.6	10.9	....	....
		5.5	66.6	53.9	35.7	10.4	....	....
		8	47.1	57.4	32.4	10.2	....	....
3	1 kg. DL-aspartic acid (unneutralized)	0	47.9	68.2	15.0	12.8	4.0	7.5
		0.5	35.8	69.7	15.1	9.0	6.2	4.5
		2	24.1	67.3	15.2	8.1	9.4	5.4
		5	18.1	66.2	17.9	9.4	6.5	6.5
4	1 kg. DL-aspartic acid (unneutralized)	0	66.5	66.0	15.7	13.0	4.4	7.6
		0.5	50.6	68.2	15.8	11.7	4.3	5.7
		2	39.2	67.6	17.2	13.4	1.8	6.5
		5	43.5	65.0	19.8	11.6	3.6	7.0
5	1 kg. DL-aspartic acid (neutralized to pH 6.9 with Na_2CO_3)	0	70.7	66.8	15.3	15.1	2.8	6.5
		0.5	60.3	67.0	15.3	14.2	3.5	7.1
		2	58.4	65.1	18.3	13.5	3.1	7.2
		5	64.2	62.7	22.5	10.9	3.9	7.2
		8	69.4	62.4	24.4	9.7	3.7	7.3

[a] Includes valeric and other higher acids.

acids. However, deamination did occur. This is in agreement with the findings of Sirotnak *et al. (18)*. Lactic acid was not observed in these rumen liquor samples.

Glucose. The D-glucose dissimilation data are shown (Table 2). The principal in vivo changes were increases in rumen propionic, butyric, and lactic acid concentrations, and decreases in rumen liquor pH and free ammonia concentration. Valeric acid concentration did not undergo any appreciable changes. A large decrease in free ammonia concentration of the rumen liquor adds confirmation to the view that ammonia utilization is enhanced by the presence of readily available carbohydrate. Increases in propionic acid concentration also were observed during the dissimilation of glucose by WCS. The presence of large amounts of glucose in the rumen appeared to affect the activity of the bacteria, because the WCS prepared 5 hr. after administering glucose attacked this substrate more vigorously and with a greater production of propionic acid than did the WCS without previous exposure to this substrate.

Maltose. In vivo maltose dissimilation (Table 3) did not result in appreciable changes in the relative proportions of VFA. Total VFA concentration increased rather markedly in most cases. This is made evident by comparing the maltose data with those of the control cow (shown in Table 1). During these trials, a relatively large increase in lactic acid occurred which, when combined with the increase in total VFA, resulted in a marked decrease in rumen pH. It will be noted that the lactic acid concentration following the administration of maltose was much greater than that from glucose in Trial 6 (Table 2). This observation agrees with the results of Robinson *et al. (15)*.

The results of the in vitro dissimilations of maltose by cell suspensions were similar to the in vivo results. The proportion of acetic acid was lower, and that of propionic and butyric acids higher, than in the case of the in vivo trials. For proper evaluation, the butyric plus higher acid fraction in the dissimilations by cell suspensions should be compared with the combined butyric and valeric acid fractions of the in vivo trials. The one major exception noted was the large increase in the propionic acid concentration of the 0-hr. WCS dissimilation with added maltose (Trial 12).

DL-lysine. The in vivo and in vitro data (Table 4) indicate that little or no VFA were produced from DL-lysine. Sirotnak *et al. (17)*, using L-lysine, obtained similar results. It will be noted that there was a decrease in total VFA in the rumen (absorption) and an increase in rumen pH. Only slight increases were noted in rumen lactic acid concentrations.

Glycine plus DL-alanine. The in vivo data provided little or no evidence of VFA production from the mixture of glycine and DL-alanine, although there was a large production of ammonia (deamination) and some production of lactic acid. The in vivo and in vitro dissimilations of the glycine and DL-alanine mixture were in close agreement, in that both indicated that the mixture, if fermented at all by the rumen microorganisms, produced negligible amounts of VFA. The uniform pH of the rumen liquor during these trials served as supporting evidence for the preceding statement. These observations verify the results of Sirotnak *et al. (17)*.

TABLE 2

In vivo and in vitro dissimilations of glucose by rumen microorganisms

Trial No.	Treatment	Sampling time	Total VFA	Acetic	Propionic	Butyric	Valeric plus higher acids	Rumen liquor	Rumen liquor lactic acid	Rumen liquor free ammonia
		(hr.)	*(μM/ml)*	*(Molar % total VFA)*				*(pH)*	*(μM/ml)*	
6	1.5 kg. glucose added in vivo	0	57.2	68.5	13.0	14.3	4.2	7.1	0.0	7.2
		0.5	50.0	63.1	16.0	18.1	2.8	6.6	2.5	4.3
		2	73.5	57.6	21.5	18.9	2.0	6.3	3.2	4.5
		5	69.5	57.9	21.5	18.5	2.1	6.9	0.0	1.0
7	1.5 kg. glucose added in vivo	0	101.5	67.8	16.6	11.9	3.7	6.6	....	....
		0.5	89.5	61.8	19.3	16.1	2.8	6.0	....	....
		2	105.0	57.6	19.4	21.2	1.8	6.0	....	....
		5	93.0	56.3	19.3	21.8	2.6	6.5	....	....
8[a]	In vitro WCS from Trial 6	0-hr.	15.0	68.0	13.5	9.1	9.4	Control, no substrate		
		0-hr.	19.5	65.0	16.8	10.4	7.3	400 *μM* glucose added		
		5-hr.	12.6	58.5	20.1	14.1	7.3	Control, no substrate		
		5-hr.	35.5	60.9	28.0	8.2	2.9	400 *μM* glucose added		

[a] Dissimilations by WCS prepared from rumen liquor obtained before (0-hr.) and after (5-hr.) adding glucose in Trial 6 above.

TABLE 3

In vivo and in vitro dissimilations of maltose by rumen microorganisms

Trial No.	Treatment	Sampling time	Total VFA	Acetic	Propionic	Butyric	Valeric plus higher acids	Rumen liquor	Rumen liquor lactic acid
		(hr.)	*(μM/ml)*	*(Molar % total VFA)*				*(pH)*	*(μM/ml)*
9	1.5 kg. maltose added in vivo	0	101.3	64.2	17.5	11.3	7.0	6.6	0.0
		0.25	85.0	61.8	18.4	14.1	5.7	6.1	11.0
		0.5	91.5	63.1	19.3	13.2	4.4	6.0	13.0
		2	140.2	62.0	18.1	16.1	3.8	5.6	4.5
		5	125.8	62.7	16.9	16.4	4.0	5.9	0.45
10	1.5 kg. maltose added in vivo	0	114.4	62.4	16.3	15.2	6.1	6.7	0.0
		0.25	100.8	60.6	19.0	14.8	5.6	6.2	8.7
		0.5	111.9	62.1	17.6	14.8	5.5	6.0	11.6
		2	129.4	59.3	18.4	17.6	4.7	5.8	5.1
		5	134.8	59.4	15.5	18.8	6.3	6.0	0.11
11	1.5 kg. maltose added in vivo	0	122.1	59.9	20.8	14.2	5.1	6.0	.....
		0.25	121.5	61.4	20.7	13.6	4.3	5.6	.....
		0.5	127.4	58.5	19.8	13.2	8.5	5.5	.....
		2	120.6	59.1	20.4	15.7	4.8	5.2	.....
		5	97.3	60.7	18.9	15.3	5.1	4.9	.....
12[a]	In vitro WCS from Trial 9	0-hr.	29.1	55.7	18.7	25.6[c]	Control, no substrate		
		0-hr.	46.2	52.9	28.2	18.9	200 *μM* maltose added		
		5-hr.	34.1	52.0	24.9	23.1	200 *μM* maltose added		
13[b]	In vitro WCS from Tria 10	0-hr.	21.1	53.5	23.0	23.5[c]	Control, no substrate		
		0-hr.	30.5	55.2	22.6	22.2	200 *μM* maltose added		
		5-hr.	34.0	53.5	23.0	23.5	200 *μM* maltose added		

[a] Dissimilations by WCS prepared from rumen liquor obtained before (0-hr.) and after (5-hr.) adding maltose in Trial 9 above.
[b] Dissimilations by WCS prepared from rumen liquor obtained before (0-hr.) and after (5-hr.) adding maltose in Trial 10 above.
[c] Includes valeric and other higher acids.

TABLE 4

In vivo and in vitro dissimilations of DL-*lysine by rumen microorganisms*

Trial No.	Treatment	Sampling time	Total VFA	Acetic	Propionic	Butyric	Valeric plus higher acids	Rumen liquor	Rumen liquor lactic acid
		(hr.)	*(μM/ml)*	*(Molar % total VFA)*				*(pH)*	*(μM/ml)*
14	1.5 kg. 4 -lysine added in vivo	0	50.4	69.6	21.4	8.5	0.5	7.4	0.0
		0.5	54.2	71.1	18.1	10.5	0.3	7.0	0.18
		2	49.9	73.4	17.8	8.5	0.3	7.2	0.0
		5	42.5	78.6	18.9	1.7	0.8	7.4	0.0
15	1.5 kg. DL-lysine added in vivo	0	38.0	64.2	19.5	13.0	3.3	6.9	0.0
		0.5	35.5	65.4	18.3	11.6	4.7	6.8	0.33
		2	33.8	66.0	18.1	12.6	3.3	7.1	0.0
		5	33.5	65.0	17.8	14.7	2.5	7.0	0.0
16[a]	In vitro WCS from Trial 17	0-hr.	17.0	68.3	22.3	9.4[b]	Control, no substrate		
		0-hr.	18.0	69.3	16.6	14.1	200 *μM* DL-lysine added		
		5-hr.	17.0	66.8	17.6	15.6	Control, no substrate		
		5-hr.	17.0	73.6	16.3	10.1	200 *μM* DL-lysine added		

[a] Dissimilations by WCS prepared from rumen liquor obtained before (0-hr.) and after (5-hr.) adding DL-lysine in Trial 14 above.
[b] Includes valeric and other higher acids.

DISCUSSION

In three trials, the in vivo and in vitro results were in close agreement qualitatively. Quantitative differences existing between in vivo and in vitro results could be owing to intraconversions among the VFA and to absorption occurring during the in vivo trials. The results of the in vivo fermentation of succinate was similar to the in vitro results reported by Doetsch *et al. (4)*. The failure to obtain any apparent fermentation of aspartic acid in vivo is attributed to the abnormal pH of the rumen contents caused by the DL-aspartic acid. The extremely high acidity (approximately pH 4.5) may have adversely affected rumen bacterial metabolism and interfered with normal dissimilation. When aspartic acid was partially neutralized to pH 6.9 with Na_2CO_3, it was attacked by rumen microorganisms and resulted in a marked increase of VFA, mainly propionic acid. The in vivo results of partially neutralized aspartic acid are similar to in vitro results of WCS reported by Sirotnak *et al. (18)*.

It is of interest to note that in vivo trials with glucose indicated that the presence of fermentable carbohydrates enhances ammonia utilization by rumen microorganisms. It was noted also that the in vivo fermentation of maltose and glucose resulted in the production of lactic acid, as well as VFA. The lactic acid reached a maximum concentration within 2 hr. after the administrations of the substrates and in 5 hr. had almost completely disappeared from the rumen. This high concentration of lactic acid may be explained by assuming that it is a normal intermediate in carbohydrate metabolism in the rumen, and that its formation is occurring at a faster rate than its dissimilation and/or absorption. Similar results concerning lactic acid production have been reported by Waldo and Schultz *(19)*.

DL-lysine or a combination of glycine and DL-alanine was not fermented to VFA by rumen microorganisms. These results agree with in vitro WCS results of Sirotnak *et al. (17)*. However, glycine plus DL-alanine was deaminated in vivo by rumen microorganisms. The identity of the compound(s) resulting is as yet unknown. Loosli *et al. (12)*, Black *et al. (2)*, and Duncan *et al. (6)* have provided evidence demonstrating the synthesis of essential amino acids by rumen microorganisms. Perhaps reactions in the rumen involving essential amino acids are entirely assimilatory in nature. Deamination processes would aid in supplying ammonia required for bacterial (McDonald, *13*), and possibly host, protein synthesis.

Observations made on rumen liquor pH values during in vivo trials indicated that the pH of the rumen liquor is directly related to the total VFA concentration of the ruminal contents. It is thought that these measurements, although not representing the precise intraruminal pH, do reflect any large changes in rumen liquor pH values.

During the in vitro trial with glucose (Trial 8, Table 2), differences were observed between the fermentation of the added glucose by the 0-hr., as compared to the 5-hr., WCS. It should be noted that increases in butyric acid occurred during both the in vivo and in vitro trials. However, with added substrate, the in vitro concentration of butyric acid by the 5-hr. WCS did not keep pace with

the in vivo concentration increases, although a considerable increase did occur. These results indicate that the in vivo administration of relatively large amounts of certain carbohydrates can alter the activity of rumen microorganisms. The effects of certain carbohydrates on lactic acid and polysaccharide production by bovine rumen bacteria have been reported by Robinson *et al. (15)* and Gibbons *et al. (8)*. Alterations may occur for one or more of the following reasons: *(a)* a bacterial population in which one or more species have increased in numbers; *(b)* an increased metabolic activity of one or more species of microorganisms; or *(c)* the establishment of a metabolic shunt pathway in the presence of excess substrate by one or more species of rumen microorganisms.

Although qualitative in vivo and in vitro results were similar, quantitative differences were noted. Variations may be the result of differential absorption of end-products, changes in pH plus other in vivo factors, and altered bacterial metabolism. These differences also could be owing to enzyme inactivation in processing cell suspensions, exclusion of protozoa, omission of important bacteria because of adherence to various material, lack of cofactors for certain reactions, assimilations, and/or utilization of some VFA, owing to the absence of absorption.

The in vitro cell suspension method appears to be a useful technique for studying toxic and/or expensive substrates; for artificially altering the chemical environment in a predictable manner and direction; for pharmacological investigations, and for pilot studies for subsequent animal experiments.

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NUTRITIONAL FACTORS IN CALF DIARRHEA[1, 2]

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SUMMARY

The influence of various components of milk-type diets on consistency of feces has been evaluated. Calves were fed modified milk (whole or skimmilk) diets to determine the effects of fat, lactose, minerals, and curd formation, individually and in all possible combinations. The presence of milk fat (3%) reduced diarrhea. Increasing the mineral content of the milk to four times normal by addition of simulated whey ash produced moderate diarrhea; increasing lactose to twice normal increased diarrhea to a lesser extent. The prevention of curd formation with sodium citrate did not increase the incidence of diarrhea. The response to minerals may involve additional factors since, under slightly different conditions in this study, the mineral content of milk diets was increased to ten times normal without causing diarrhea.

Diarrhea is a primary concern in raising diary calves. Not only is diarrhea a major factor contributing to the high rate of calfhood mortality, but it lowers resistance to other diseases, slows the growth rate, and reduces the efficiency of feed utilization. No doubt, infectious agents are often involved; however, nutritional (and managemental) factors also deserve attention. Phillips *et al. (16)* reported that vitamin A and members of the vitamin B complex were beneficial as preventives of diarrhea in milk-fed calves, but other studies *(11, 15)* failed to substantiate this work. Since the advent of whole-milk replacers, a knowledge of the possible influence of the various constituents of milk and milk products on the diarrheic nature of the diet has become of greater practical and fundamental importance. Wing *et al. (21)* suggested that the diarrhea produced during the feeding of a whole-milk replacer emoposed primarily of dried when product may have been the result of the high mineral content in this diet and/or of the absence of curd formation in the stomach. However, the specific influence of these factors, or the other major constituents of milk, on calf diarrhea has not been investigated extensively.

The present study was designed to examine further the relation of certain components and characteristics of milk and milk products to the characteristics of calf feces. Two experiments were employed: a preliminary experiment (I), to explore the contribution of lactose, minerals, butter oil, casein, lactalbumin, and curd formation to the diarrheic property of the diet of calves, and a subsequent experiment (II), to evaluate critically the influence of lactose, minerals, milk fat, and curd formation as independent and interacting factors affecting color and consistency of feces.

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EXPERIMENTAL PROCEDURES AND RESULTS

Experiment I

Procedure. Preliminary tests were made to obtain a general appraisal of the effects of certain milk-type diets on consistency of feces. Seventeen calves from the Iowa State College dairy herd were used. Details of the design of each succeeding phase of these tests were determined in part by the results of the preceding phase. Calves remained with their dams for three days, then were moved to a calf barn and, in most cases, were placed in individual wooden pens provided with water and wood-shavings bedding. Calves used in the test of curd tension were housed in pens having metal-screen bottoms and metal and wood sides.

The basal diets employed were whole milk, reconstituted skimmilk,[4] and semisynthetic diets, supplemented during most of the experiment with 100 mg. of a commercial trace-mineral mixture, 500 U.S.P. units of vitamin D_2 in irradiated yeast, and 50 mg. of chlortetracycline as Aurofac D[5] per calf daily. All supplements were stirred into the milk (about 37° C.) immediately before each feeding. Diets were administered via nipple-pail twice daily. The diets (Table 1)

[4] Spray-process nonfat dry milk solids.

[5] Supplied by Fine Chemicals Division, American Cyanamid Co., Pearl River, N. Y.

TABLE 1

Influence of modified and semisynthetic milk diets on fecal consistency

(Experiment I)

Supplementation of basal diet	Basal diets				
	Whole milk	10% Dried skimmilk (reconstituted)	2.4% Lactalbumin suspension	2.4% Casein suspension	14% Whey product suspension
	Rate of feeding *(% body weight)*				
	8	10	8	8	10
	Average scours index[a]				
None		0.2(2)[b]			2.4(9)
Minerals I (MI), 2.1%[c, d]	1.0(7)				
Minerals II (MII), 2.1%[d]	1.0(5)	0.6(2)			
Lactose (L), 5.0%[d]	0.6(7)	1.3(11)			
Butter oil (BO), 3.0%					1.8(2)
KOH, to neutralize					2.4(5)
L + MI[e]	0.2(8)				
L + MII	0.8(18)	2.0(16)	0.8(10)	1.2(4)	
L + BO		0.9(10)			
BO, 2.8% + lecithin, 0.2%					1.7(6)
BO + MII + L		0.6(8)	0.7(5)	0.9(3)	
L + MII + lactalbumin, 1.0%	0.7(5)				
L + BO, 2.8% + lecithin, 0.2%		0.2(3)			

[a] Scours index = fecal-consistency evaluations on the scale 0 to 4, denoting normal feces through watery diarrhea. Observations included in the average began 24 hr. after diet administration and continued to a maximum of six observations (made twice daily) per trial.

[b] Numbers in parentheses represent number of trials in which each diet was fed.

[c] Higher levels, up to 6.3%, were used in several trials.

[d] Amounts added provided a total daily intake of these nutrients equal to or slightly greater than the amount in the whey-product diet.

[e] Amounts used in the last seven diets are the same as shown above, unless otherwise indicated.

were formulated to imitate, in various respects, the composition of a reconstituted whey product[6] which was found to be rather consistently laxative. The apparent inconsistency in levels of feeding of diets (shown in Table 1) was the result of an attempt to equalize certain of the nutrients and, at the same time, provide energy sufficient for maintenance requirements, at least. When lactose and minerals were added to the milk, the resulting levels of intake of these nutrients were equal to or slightly above the levels resulting when whey product was fed.

The mineral levels in reconstituted whey product (2.04%), compared to the amount in whole milk (0.7%), prompted trials in which whole milk was supplemented with a mineral mixture (Minerals I)[7] containing the principal elements of milk ash. Minerals I subsequently was replaced by Minerals II,[8] which more closely simulated whey ash. During several of the first trials, mineral-supplement levels were increased gradually up to 6.3% of the milk fed. The butter oil was homogenized into the diet at 2,000-lb. pressure. Potassium hydroxide was added to certain whey diets to increase the pH from about 4.7 to 7.0. The curd tests were made on Holstein milk; portions of the high- and low-curd milks were separated soon after the afternoon milking. Duration of the trials usually was between two and six days, but in some instances extended to periods of over 2 wk.

Observations on the color and consistency of feces were made twice daily (A.M. and P.M.) by manual stimulation of defecation. Body weights and temperatures also were obtained daily.

Results. The average consistency of feces of calves on the various diets is shown (Tables 1 and 2). Minerals added to whole milk at levels of from one to nine times the amount normally present in milk apparently had no serious effect on fecal consistency. Except for a few occasions, at the highest levels of minerals, these diets were readily consumed. Only two calves of the 12 on high-mineral diets showed appreciable diarrhea, and continued feeding of these diets for

[6] Dried whey products was furnished by Western Condensing Co., Appleton, Wis. Its proximate percentage composition was: nitrogen-free extract, 57.6 (lactose, 46); protein, 17.2; ash, 14.6; fat, 0.4, and fiber, 0.0.

[7] Minerals I contained 300 g. $Ca_3(PO_4)_2$, 300 g. KCl, and 400 g. NaCl.

[8] Minerals II contained 356 g. $CaHPO_4$ (added as an equivalent amount, 450 g., of $CaHPO_4 \cdot 2H_2O$), 360 g. K_2HPO_4, 175 g. $MgSO_4$, and 390 g. NaCl.

TABLE 2

Effect of curd tension (CT) on the laxativeness of milk diets

(Experiment I)

Supplementation of basal diet	Basal diets[a] *(10% of body weight)*				
	Whole milk			Raw skimmilk	
	Noncurd	Low CT	High CT	Low CT	High CT
	Average scours index[b]				
(None)	0.4(3)			0.3(2)	
Lactose, 5% + minerals, 2.1%		1.7(3)	1.6(6)	2.1(5)	1.7(4)

[a] Noncurd milk (from one cow) did not form curd in vitro. Low CT milk averaged about 30 g. of curd tension. High CT milk averaged about 60 g. of curd tension.

[b] Scours index = fecal-consistency evaluation made twice daily on the scale 0 to 4, denoting normal feces through watery diarrhea.

periods of over 2 wk. was generally accompanied by firm feces. After prolonged feeding of whole milk, containing abnormally high amounts of minerals, most of the calves exhibited long, rough coats, unthriftiness, and occasional tremors. Lactose added at 5% of the whole-milk diets, with or without mineral supplementation, seemed to have no influence on consistency of feces; similarly, lactalbumin had no consistent effect.

With reconstituted skimmilk as the basal diet, lactose supplementation produced moderate looseness, and lactose plus mineral supplements resulted in very loose feces. The latter was true also when lactose and minerals were administered in either high- or low-curd tension milk (Table 2). Inclusion of butter oil, with or without lecithin, in the high-lactose or high-lactose-plus-mineral diets markedly reduced the diarrheic effects.

Feeding reconstituted whey product consistently produced a greenish-black, watery diarrhea, even when the pH was raised to approximately 7 before feeding. Diarrhea was even more severe than that observed after the feeding either of skimmilk or of lactalbumin suspension supplemented with lactose and minerals. Butter oil, with or without lecithin, homogenized into the whey-product diet, tended to delay the appearance of the diarrhea several days. None of the semisynthetic diets containing lactalbumin or casein was consistently diarrheic.

Chewing on the pens and eating the wood-shavings bedding were common after calves were from 5 to 6 wk. of age. Though calves changed little in body weight during this study, body temperatures and general appearance indicated that no serious disease conditions were involved.

No noticeable difference in consistency of feces was evidenced from feeding calves milk of different curd tensions (Table 2). Moderate diarrhea resulted when raw skimmilk and whole milk were supplemented with lactose and minerals at the two levels of curd tension tested. Calves used in this study acquired chronic looseness of feces which resisted return to normalcy; consequently, some of the anticipated tests were not made. The apparent discrepancy between the results presented in Table 1 and those in Table 2, for feeding a whole-milk diet supplemented with lactose and minerals, appears to be related to the type of pens (and bedding) used. The aforementioned group may have received an antidiarrheic effect from consuming some of the shavings used for bedding (see Discussion).

Experiment II

Procedure. The design of this experiment was based primarily upon the results from preliminary studies. A factorial trial was employed, using an 8×8 quasi-Latin square *(3)*, in order to test more critically some of the factors explored during preliminary work.

Four factors (fat, lactose, minerals, and curd formation) were tested separately and in all possible combinations, requiring a total of 16 diets. Eight Holstein male calves were assigned at random to ration sequences (also established randomly) composed of eight diets. This permitted the testing of each diet by four calves and a total of 64 individual trials.

Diets were tested during four-day periods, with minimal three-day adjustment periods of whole-milk feeding allowed before subsequent test feedings. When severe diarrhea occurred, the scheduled feedings were discontinued. Scheduled dietary sequences were continued as soon as feces maintained normal firmness for two days.

At four days of age, calves were removed from their dams and placed in new, individual calf pens. These pens were fitted with expanded-metal-screen bottoms and had metal partitions and tile walls, minimizing the consumption of extraneous material by the animals. A basal whole-milk diet was fed for from four to 11 days of age at 8% of body weight, in the manner used in the preliminary experiment. Experimental feeding was begun at 11 days of age, at 8% of body weight (adjusted weekly for weight increases). Trace minerals[9] and a vitamin-antibiotic mixture[10] were added to all diets after four days of age.

The milk fed was a mixture composited after the afternoon milking of equal amounts from five Holstein cows, and contained about 3.1% fat. A portion of this was separated for the skimmilk diets (testing about 0.02% fat). Amounts needed for morning feedings were stored overnight in a refrigerator. For the test of fat on fecal consistency, the results of diets with whole milk were compared to those containing skimmilk. The amounts of lactose (5%) and minerals (2.1%) used were selected because of the effects observed in preliminary studies (see Table 1). For the test of curd formation, 3 ml. of a 60% solution of sodium citrate (dihydrate) was added per pound of milk. In tests with young fistulated calves, this procedure prevented curd formation, as determined by palpation of the abomasal contents through the rumen wall. The effectiveness of sodium citrate also was verified in diets containing milk with or without lactose and minerals at levels used in this experiment.

Consistency and color of feces were noted twice daily by observing the feces on the concrete floor beneath the floor grating. Body temperatures were recorded daily and weights weekly.

Results. The mean fecal-consistency evaluations for the four calves fed each diet are presented (Table 3). It is noted that the three most diarrheic diets contained both lactose and minerals and had an average effect of moderate laxation. Feces usually were dark green in color, sometimes almost black. A greenish effect was especially common during mineral supplementation. In most instances, calves were normal in fecal consistency at 11 days of age, when experimental feeding was scheduled to begin, and usually recovered from any laxation of preceding trials during the three-day recuperation period.

Since the distribution of the scours-index values was approximately Poisson, the data were transformed as follows:

$$\sqrt{\text{Scours index} + 0.4}\,.$$

[9] Eight ml. added to the milk of each calf per feeding of a mineral solution composed of $MgSO_4 \cdot 7H_2O$, 1,355.0 g.; $FeC_6H_5O_7 \cdot H_2O$, 133.0 g.; $MnSO_4 \cdot H_2O$, 33.8 g.; $CuSO_4 \cdot 5H_2O$, 8.8 g.; KI, 1.5 g., and $Co(C_2H_3O_2)_2 \cdot 4H_2O$, 1.0 g., made up to 4,000 ml. with distilled water.

[10] One capsule per calf daily, containing 2.3 g. of the following mixture: chlortetracycline HCl (crystalline), 25 g.; irradiated yeast (9,000 USP units/g), 8.3 g., and vitamin A palmitate feed supplement (5,000 USP units/g), 250 g.

TABLE 3

Mean fecal-consistency observations for calves fed modified milk diets

(Experiment II)

Supplementation of basal diet	Basal diets[a]	
	Whole milk	Skimmilk
	Average scours index[b]	
(None)	0.3	0.7
Minerals II,[c] 2.1%	0.8	1.2
Lactose, 5.0%	1.0	0.6
Sodium citrate, dihydrate, 0.40%	0.3	0.8
Citrate+ Lactose[d]	0.3	0.8
Citrate + Minerals II	0.6	1.4
Lactose + Minerals II	2.0	1.7
Citrate + Lactose + Minerals II	1.0	1.9

[a] Fed at 8% of body weight.

[b] Average of fecal-consistency evaluations made twice daily on the scale 0 to 4, denoting normal feces through water diarrhea. Observations included in the average began 24 hr. after diet administration and continued to a maximum of six observations per trial.

[c] Composed of mixture of 356 g. $CaHPO_4$ (added as its equivalent, 450 g., $CaHPO_4 \cdot 2H_2O$); 360 g. K_2HPO_4; 175 g. $MgSO_4$. and 390 g. NaCl.

[d] Rate of supplementation is the same in combinations as when added singly.

The analysis of variance of the transformed data indicates significant influences of milk fat and lactose at $P = 0.05$ and of minerals at $P = 0.01$. Table 4 gives the mean main effects and interactions both for the original and for the transformed data. Minerals and lactose, respectively, were found to have a positive effect toward producing laxation; whereas, butterfat had a negative, or antidiarrheic, action. These effects were independent of the effects of other factors. The absence of curd formation in the abomasum, as produced by the action of sodium citrate, did not appear to influence consistency of feces.

TABLE 4

Estimate of effects of nutritional factors on fecal consistency

Factor	Mean effects	
	Scours index[a]	$\sqrt{\text{Scours index} + 0.4}$[b]
Fat (F)	−0.401	−0.177[c]
Curd-formation (C)	−0.214	−0.094
Lactose (L)	0.338	0.137[c]
Minerals (M)	0.808	0.335[d]
F × C	−0.245	−0.103
F × L	−0.224	0.083
F × M	−0.141	−0.014
C × L	−0.130	−0.042
C × M	−0.078	−0.030
L × M	0.172	0.075
F × C × L	−0.104	−0.084
F × C × M	−0.069	0.047
F × L × M	0.250	0.049
C × L × M	0.264	0.102

[a] Scours index = fecal-consistency values based on the scale 0 to 4, denoting normal feces through watery diarrhea.

[b] Standard error of the mean main effects and two-factor interactions = 0.066, and of the three-factor interactions = 0.076.

[c] Significant at $P = 0.05$.

[d] Significant at $P = 0.01$.

Feces were voided by normal calves at approximately 24- to 36-hr. intervals; in very young calves much longer intervals sometimes were observed. Weight changes during the course of this experiment were small; however, calves appeared to be vigorous at all times.

DISCUSSION

Milk fat was the only factor investigated which significantly reduced the laxativeness of milk diets. Studies of Wing *et al. (21)* suggested this role of fat in calf diets, an effect which had been observed earlier with white rats fed high melting-point triglycerides *(20)*. Gullickson *et al. (9)* found that calves fed diets containing butter oil were less affected by scours than those fed diets containing oils having lower melting points. The cause for the costive action of fat has not been determined.

It seems possible that the effect of fat in reducing the diarrhea associated with high levels of minerals in the diet may be related to *(a)* the effect of fat in increasing mineral utilization *(2, 12)*, *(b)* conjugation of the fatty acids liberated from the dietary fat with the dietary minerals, reducing osmotic pressure by a decrease in molecular entities, *(c)* complexing of bile acids with fatty acids, and *(d)* providing a protective coating to the mucosa, reducing sensitivity of the intestinal tract.

The suggestion of a diarrheic effect from lactose, observed both in the preliminary and in the final study, agrees with previous reports *(1, 8, 17)*. Increased acid production in the intestinal tract has been suggested *(6, 14)* as the cause of the digestive disturbance. Should this be correct, the rapidity of the onset of diarrhea from high-lactose diets suggests a correspondingly rapid conversion of bacterial flora to aciduric types. A hydragogue effect also has been suggested as the mode of action of lactose in increasing intestinal motility *(7)*.

A high level of minerals seems to be a definite factor in the cause of much of the diarrhea which occurred during these studies. Reducing the mineral content of whey by dialysis prevented diarrhea in rats *(5)*, and adding the dialysate back to the dialyzed whey restored its laxative property. Though no direct evidence is available to explain the phenomenon of mineral laxation, the following seem to be possibilities: *(a)* Inorganic ions may have a direct hydragogue effect which is considered the mechanism of certain mineral laxatives, such as magnesium sulphate. The salts of magnesium and the sulphates and phosphates of sodium and potassium are effective in producing increased fluid retention in the intestine through osmosis *(18)*. Distention of the tract by the large liquid content provokes contraction and increases passage of tract contents. *(b)* Minerals may stimulate indirectly the secretion and/or excretion of bile, which is recognized as possessing laxative action *(18)*. The greenish appearance of feces characteristically observed during the feeding of high levels of minerals may be the result of the excretion of large amounts of bile *(19)*. The presence in the feces of this pigment might be the result of an abnormally large amount of excreted bile or of the incomplete transformation of biliverdin to stercobilin,

the principal pigment of normal feces. It has been shown that magnesium sulphate, a constituent of the mineral supplement used in these experiments, promotes evacuation of bile from the gall bladder by dilation of the bladder sphincter *(10)*. It seems possible, then, that the role of fat in reducing, or preventing in some cases, mineral laxation may be owing to the complexing of fatty acids with the bile acids in the formation of choleic acids *(10)* and/or other compounds. The bile thereby might lose its laxative effect. *(c)* Minerals also might inhibit lactose hydrolysis and galactose utilization and indirectly induce intestinal hypermotility. Such an effect has been claimed for calcium chloride *(4)*. *(d)* Since fatty acids may activate the passage of secretin into the blood stream *(10)*, complexing of minerals with fatty acids possibly might impair this process and disturb pancreatic digestion.

The absence of an adverse effect from feeding curd-inhibited diets is contrary to the results of others *(1, 13)*. Blaxter and Wood *(1)* state that when clot formation is inhibited, scours inevitably follow. However, the absolute effect of coagulation was not ascertained in their work, since the diets used either were abnormally low in casein or contained gelatin; thus, an adequate control diet (containing like ingredients) was not employed.

The contrasting effect of added lactose and minerals in whole milk, fed to calves in different type pens, might be attributable to the wood-shavings bedding used in the earlier studies, as no other aspect of these trials differed greatly. A costive effect may have been produced by tannin in the shavings eaten by the calves housed in pens bedded with shavings. Tannic acid tends to arrest diarrhea by allaying intestinal inflammation through an astringent action *(18)*. Whether the amount of tannin present in the shavings was sufficient to have a significant effect is not known. It is also possible that the effect of shavings was partially or entirely physical in nature. The shavings may have absorbed moisture or, merely by mixing with the residue from digestion, may have altered the consistency of feces. It must be recognized also that although bedding appeared to be the only major difference to which the variations in diarrhea (other than those owing to diet) could be attributed, other possible differences of environmental and genetic nature can not be ignored completely.

Since the feeding of a lactalbumin suspension containing lactose and minerals to simulate whey product did not demonstrate any consistent laxative effect, it might be speculated that the composition of the whey product used in this study differed in some of the following respects from the simulated whey-product diet: *(a)* A lactose-lactalbumin complex may be present as a result of the browning reaction occurring during the heat treatment in processing of some whey. *(b)* The ratio of α- to β-lactose may differ, in the whey product employed, from that in the commercial lactose used. *(c)* Other components present in relatively small amounts in the whey product may be of significance. The laxative effect of whey product, then, seems to be owing to factors other than its protein and appears to be partially, but not entirely, attributable to its high mineral content. Lactose also may be involved, but probably to a lesser extent. The absence of

curd-forming potential and low pH appear unrelated to the laxativeness of the whey-product diets.

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FERTILITY RATES OF COWS BRED FOLLOWING ESTROUS CYCLES OF VARYING LENGTHS[1, 2]

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SUMMARY

This study shows no practical reason for delaying service because of estrous cycle irregularity, if the cow is otherwise normal and the cycle is at least 17 days. The conception rates for cycles of less than 17 days were 34% ($P < 0.01$) among normal cows not bred at the previous estrus, as compared with 50% for all services in this grouping. Similarly, those bred following intervals of 26-27 days were generally below average, but were not significant. Conception rates among otherwise normal cows which failed to conceive at the previous estrus were significantly lower for cycles of 20–22, 32–34, and 35–37 days, and were significantly higher for cycles over 50 days in length. The average was 40% for all services in this cow-group. Among otherwise abnormal cows not bred at the previous estrus, conception rates were significantly lower for estrous intervals of 2–16 (19%), 26–28 (21%), and 29–31 (12%) days in length. This cow-group averaged 29%. Only cycles occurring 50–100 days after a previous breeding had significantly higher conception rates in the group of abnormal cows (26 *vs.* 21%). Abnormal cows repeating for service anytime within 37 days profitably might have been withheld from service (19–22 days, a possible exception), because the chances for conception were one-third greater for cycles exceeding 43 days in length. The study involved 19,369 services following estrous cycles of known lengths. The data were from one herd of Holsteins for a 25-yr. period.

It is frequently recommended that cows not be serviced following estrous cycles of irregular length. This generally means cycles occurring outside an interval of 18–25 days. Since over 40% of all estrous cycles can be expected at intervals shorter or longer than 18–25 days, a large number of cows becomes involved *(5)*. The question arises as to the effect on the chances for conception when cows are serviced at the termination of an irregular cycle. Ellenberger and Lohman *(3)* observed from 274 cycles that conception rates were 40, 55, and 48%, respectively, for cycles shorter than 17, 17–25, and over 25 days. Moeller and VanDemark *(7)*, using 4,885 repeat-service cycles from artificial breeding, observed 180-day nonreturn rates of 34, 49, 45, 50, and 54%, respectively, for cycles of 2–17, 18–25, 26–35, 36–50, and 51–72 days after the previous insemination. Only the 2–17 day group differed significantly from the mean of all. Flerchinger and Erb *(6)* and Olds and Seath *(9)* have made similar observations.

From previous work *(3, 6, 7, 9)*, it was not possible to determine what effect genital abnormalities and previous service had on conception rates. An added bias in artificial breeding data is that cows with long returns to service are more likely to be sold without rebreeding and thus appear as a nonreturn. This would

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tend to make nonreturns for repeat services higher for intervals over 40 days. Some evidence for this is suggested from the data of Moeller and VanDemark *(7)* and Flerchinger and Erb *(6)*.

The present study was undertaken to compare conception rates between nonservice and repeat-service cycles of variable lengths, for normal and abnormal cows.

MATERIALS AND METHODS

Breeding records from the Carnation Milk Farms' Holstein-Friesian herd at Carnation, Washington, were used. These records were supervised by a single herdsman and extended from 1926 to 1950. Resident veterinarians recorded results of clinical examinations on individual cow-history cards. On the opposite side of the card the herdsman recorded estrus, service sire, and calving dates. Methods of housing and general management were quite constant during the experimental period. Conditions were such that all cows could be observed readily during the day, whether on pasture, in box stalls, or in exercise pens. Although observation at night was not ideal, some cows in the herd were milked four times daily throughout this period and all were milked at least three times daily. This reduced the time cows were not under observation. Natural service was used before 1940, and artificial service with semen containing no antibiotics was used exclusively after 1940. Erb *et al. (5)*, in a previous study of these data, found no significant differences between natural and artificial breeding. Therefore, the two periods were not considered separately in this study.

Morrison and Erb *(8)* previously have reported the reproductive capacities and sterility rates among cows in this study.

The data were coded on International Business Machine cards by date of occurrence for estrus, service, service sire, reproductive abnormality, calving date, and type of birth. A separate card was used for each event occurring throughout the lifetime of the cows. By creating a time sequence covering the experimental period, it was possible numerically to identify each date and to calculate the intervals electronically by simple subtraction.

Estrous cycles were classified according to (1) normal, (2) abnormal, (3) service, and (4) nonservice. A cow which had no reproductive abnormality observed during the reproductive period (after calving to the next calving or until disposal) was termed normal. Normalcy of estrous cycles or normalcy of calving was not involved when characterizing cows as normal. Such calving abnormalities as twinning, abortions, still-births, and retained placentae were found to have no significant influence on estrous cycle distributions, provided the reproductive tract was normal subsequently *(5)*. Only those abnormal calvings which were associated with later reproductive abnormalities were in the abnormal group of reproductive periods. Therefore, the characterization of normal cows was based on the absence of gross clinical symptoms, not involving calving or length of the estrous cycles, during the reproductive period. Undoubtedly, some cows characterized as normal were not, because of relatively minor and unobserved cases of infection in the reproductive tract.

Estrous cycles which commenced with breeding at the previous estrus were classified as service (repeat-service) cycles. Those when service was not involved at the previous estrus were classified as nonservice cycles. This classification was considered important, to eliminate partially the effects of early embryonic mortality *(11)* from one group of cycles. The influence of service sire was not completely removed from the nonservice cycle group, because occasionally a cow was not bred at the next estrus subsequent to breeding. The next estrus in these instances was classified as a nonservice cycle.

The conception rate was determined for each cycle length within type of cycle. As shown (Table 1), these were combined into subclasses in order to have sufficient services upon which to estimate conception rates. The 1 and 5% confidence limits *(10)* were calculated for each subclass mean and compared with the mean observed for each type of cycle grouping (see footnote, Table 1).

RESULTS AND DISCUSSION

There were 19,369 services (Table 1) following cycles of known lengths. The study averaged 50, 40, 29, and 21%, respectively, for normal nonservice, normal service, abnormal nonservice, and abnormal service cycles. These values are somewhat lower than those previously reported *(8)*, because all services to infertile cows are included, and because cows conceiving at the first estrus after calving would not appear in these summaries.

Among breedings following normal nonservice cycles, only the 2–16 day group had a conception rate significantly below the average for all normal nonservice cycles (Table 1). Greater differences were found among the normal service cycles. Normal service cycles 50–100 and over 100 days in length had signifi-

TABLE 1

Conception rates following service at cycles of varying lengths

Days in estrous interval	Normal cows				Abnormal cows			
	Nonservice cycles		Service cycles		Nonservice cycles		Service cycles	
	Services	Conceived	Services	Conceived	Services	Conceived	Services	Conceived
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
2–16	118	34[a]	89	30[b]	157	19[a]	146	16
17–19	326	48	491	35[b]	389	30	595	19
20–22	1,175	50	1,548	36[a]	1,326	30	1,766	21
23–25	571	53	645	39	651	32	892	17
26–28	137	41	192	35	156	21[b]	267	15[a]
29–31	49	53	140	32[b]	74	12[a]	132	15
32–34	48	40	103	23[a]	60	33	128	16
35–37	58	46	149	31[b]	77	31	176	15[b]
38–43	294	52	510	42	346	32	582	23
44–49	242	46	315	42	254	29	400	26[b]
50–100	476	54	787	49[a]	564	27	927	26[a]
Over 100	114	51	201	59[a]	170	24	356	26[b]
Total	3,608	50	5,170	40	4,224	29	6,357	21

[a] Confidence limits outside of group mean at 1% level of probability.
[b] Confidence limits outside of group mean at 5% level of probability.

Subclass confidence limit = mean $\pm$ [a]0.01 or 0.05 $\sqrt{\frac{p(1-p)}{n}}$.

cantly higher conception rates, and the 2–16, 17–19, and 20–22 day cycles were significantly below average as compared with all normal service cycles. This compares favorably with earlier observations for repeat service cycles *(6, 7)*.

The 26–34 day normal nonservice cycles as a group averaged 43% ($P>0.10$), with considerable variation between the 26–28, 29–31, and 32–34 day subclasses (shown in Table 1). In comparison, the conception rates for 29–31, 32–34, and 35–37 day normal service cycles were significantly lower than the average for all normal service cycles.

Conception rates for abnormal nonservice cycles were much lower than those for normal nonservice cycles. Although at a lower level the trends were similar. The 2–16, 26–28, and 29–31 day cycles were significantly lower than the average for all abnormal nonservice cycles. Conception rates for abnormal service cycles were generally below the group average, except for intervals exceeding 43 days ($P>0.01$). In this group, the average conception rate was 26% for intervals of 44–49, 50–100, and over 100 days. It is of interest that the conception rates for the longer service intervals (normal and abnormal) were comparable with the conception rates of their respective nonservice cycles, but were considerably lower for all other groups. This suggests that time may be an important factor in correcting temporary infertility in repeat-breeding cows.

Delaying service one estrous cycle among repeat-service cows may be economically important when they repeat for service within 35 days. Further study of this problem is of practical importance. There also may be a basic explanation, because Black *et al. (1, 2)* have observed that repeat-breeding cows have more resistance to infection than first-service heifers and cows. These observations suggest that such uteri do not offer a suitable environment for implantation, or that they interfere with sperm survival during the sperms' passage.

From these data, and the data of others *(3, 6, 7, 9)*, there appears no practical reason for delaying service because of estrous cycle irregularity, provided the cow is otherwise normal. One might consider delaying service following cycles of 16 days or less (normal cows), because little time would be lost in waiting for the next cycle. Such cows may be temporarily cystic and require prompt treatment to avoid permanent development of the condition. Abnormal cows repeating for service anytime within 37 days profitably might be withheld from service (19–22 days, a possible exception), because the chances for conception are over one-third greater for abnormal service cycles exceeding 43 days. These chances may be even greater, because some cows in this study were withheld from repeat service; thus, when again rebred, their data would appear in the nonservice summary.

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EFFECT OF PULVERIZED LIMESTONE AND DICALCIUM PHOSPHATE ON THE NUTRITIVE VALUE OF DAIRY CATTLE FEED [1]

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SUMMARY

The effect of pulverized limestone and dicalcium phosphate supplements on the nutritive value of dairy cattle feed was studied, with 12 dairy heifers receiving either an all roughage or a mixed ration. Results of these experiments indicate that pulverized limestone when added to both all roughage and mixed rations for dairy heifers decreased the digestibility of both protein and energy. Similar amounts of dicalcium phosphate did not have this effect. The feeding of dicalcium phosphate along with pulverized limestone decreased the depressing effects of the limestone. These results would indicate that the feeding of calcium in excess of requirements, particularly in wide calcium: phosphorus ratios, may reduce the digestibilities of protein and energy. These results further indicate a need for additional work on the effects of different levels of calcium and phosphorus on feed utilization.

Feedmen and dairy husbandry research workers believe generally that the calcium content of the ration usually is adequate to satisfy the requirements of cattle when legume forage is fed, but that it may be inadequate when most of the forage is nonlegume. They advocate that calcium or calcium-phosphorus supplements such as pulverized limestone, steamed bone meal, defluorinated rock phosphate, or dicalcium phosphate, or a combination of two of these, be added to commercial dairy rations at a rate of 1 or 2% of the grain mixture.

Some researchers oppose calcium supplementation of dairy feeds. These workers say that contrary to common beliefs and practices, cows at average to moderately high levels of milk production, fed liberal allowances of fair-quality roughages and enough concentrate mixture to balance the ration, do not require added calcium supplements. In their opinion, the practice of forcing cattle to eat calcium supplement with all their grain not only is unnecessary and uneconomical but also undesirable from the standpoint of good nutrition. This idea is contrary to the general belief that mineral supplementation, while it may be costly, never harms the animal or affects the use of the ration, when fed within the limits of tolerance.

Relatively little work appears to have been done to determine if supplemental calcium does affect the nutritive value of the ration adversely. Møllgaard *et al. (3)* in 1941 found that the digestion coefficient of organic matter in A.I.V. silage was lowered when calcium carbonate was added to the ration. The decrease was as high as 15.2%. These results were obtained in metabolism experiments with cows. Axelson *et al (1)* from two digestion trials in 1949 with wethers, concluded that there was no beneficial effect owing to the addition of calcium carbonate.

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The authors became interested in this problem when they failed to increase the digestibility of grass silage by neutralizing the acid with pulzerized limestone. Digestibility of both protein and energy were decreased, rather than increased. The results with silage posed the important question of the harm which addition of limestone to the ration might do. This study was undertaken to obtain quantitative information on the effects of supplemental calcium on the digestion and utilization of protein and energy by dairy cattle.

EXPERIMENTAL PROCEDURE

The experiments to determine the effect of pulverized limestone and dicalcium phosphate on the nutritive value of dairy cattle feed were carried out during the winter and spring periods between 1950–51 and 1954–55. Twelve dairy heifers between 18 and 24 mo. of age were used as experimental subjects.

In the 1950–51 season, six heifers were used in three sets of exploratory digestion experiments, using Ladino clover–bromegrass silage preserved with molasses, timothy silage preserved with sulfur dioxide, and timothy silage preserved with molasses. Silage made up the entire ration, with the exception that half of the animals fed each silage received 100 g. of pulverized limestone per day. All animals were given a cobalt supplement and allowed free access to plain salt (NaCl) bricks.

During the 1951–52 season, a more comprehensive series of experiments was carried out with a grass-legume silage preserved with molasses. Two levels of limestone were fed in addition to the basal ration. The plan of this work is given (Table 1). The animals were given supplementary cobalt as well as free access to plain salt (NaCl) bricks.

TABLE 1

Plan of experiments with grass-legume silage preserved with molasses (1951–52)

Animal No.	Period 1	Period 2	Period 3
11	Silage	Silage plus 50 g. limestone	Silage plus 100 g. limestone
17	Silage plus 50 g. limestone	Silage plus 100 g. limestone	Silage
18	Silage plus 100 g. limestone	Silage	Silage plus 50 g. limestone

In the 1954–55 experiments,[2] the rations fed consisted of mixed grass hay and a 16% crude protein concentrate mixture, 50-50 by weight. In this experiment, both pulverized limestone and dicalcium phosphate were fed as indicated in the plan of the experiment (Table 2). The mineral supplementation is given as a percentage of the concentrate portion of the ration. In this experiment, the animals were allowed free access to trace-mineralized bricks.

The procedure and methods generally used in the laboratory were followed. These consist of a 10- to 14-day preliminary period, during which the animal

[2] The Shea Chemical Co., Adams, Massachusetts, furnished the dicalcium phosphate and pulverized limestone used in the 1954–55 experiments.

TABLE 2

Plan of experiments with mixed ration

(1954–55)

Animal No.	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6
G4	2% limestone	2% limestone + 2% dicalcium phosphate	2% dicalcium phosphate	0-minerals	1% limestone	1% dicalcium phosphate
G5	2% limestone + 2% dicalcium phosphate	2% dicalcium phosphate	0-minerals	2% limestone	1% limestone	1% dicalcium phosphate
H35	2% dicalcium phosphate	0-minerals	2% limestone	2% limestone + 2% dicalcium phosphate	1% dicalcium phosphate	1% limestone
H36	0-minerals	2% limestone	2% limestone + 2% dicalcium phosphate	2% dicalcium phosphate	1% dicalcium phosphate	1% limestone

becomes adjusted to the kind and amount of the ration being evaluated. A collection period of 10–14 days follows. During this period, the feed eaten and the separately collected urine and feces excreted are carefully weighed, sampled, and analyzed. At the end of the collection period, each animal then is put in the respiration chamber for two consecutive 12-hr. periods, during which the heat and methane production are measured.

Although the methods of chemical and caloric analyses are those conventionally used, and given in the latest edition of A.O.A.C., some changes have been made in the sampling and in the method of combustion of the silages and feces. The protein analyses of the excreta, as well as the silages, were made on the fresh material. The heat of combustion of the silages and feces was determined on the fresh material according to the method given by Colovos *et al. (2)*, using an adiabatic calorimeter. However, the urine was dried in a vacuum oven at 60° C. before it was burned.

RESULTS AND DISCUSSION

The protein and energy content of the silages, hay, and grain fed during the experiments is shown (Table 3).

TABLE 3

Composition of silages, hay, and grain as fed

Feed	Moisture	Protein	Gross energy
	(%)		(Cal/g)
		1950–51	
Ladino-brome molasses silage	76.30	3.164	1.0372
Timothy-SO_2 silage	64.68	2.610	1.8019
Timothy-molasses silage	65.32	2.559	1.7599
		1951–52	
Mixed grass-legume-molasses silage	72.65	3.894	1.3374
		1954–55	
Hay	8.37	6.387	4.4251
Grain	9.73	16.819	4.4637

The effect of pulverized limestone on the digestibilities of the protein and the energy of the grass silages, used in the experiments of 1950–51 and 1951–52, are shown (Tables 4 and 5). When 100 g. of pulverized limestone were added to the all-silage ration of the dairy heifers, it caused a depression in the digestibility of the protein and the energy in all three silages fed to the animals during the 1950–51 experiments.

When the experiment was repeated in 1951–52 with three dairy heifers, with each animal being on a 0-, 50-, and 100-g. pulverized limestone daily intake, along with grass silage as the sole ration, the results of the previous year were confirmed. The addition of 100 g. of limestone again caused a significant depression in the digestibility of both protein and energy. There was not a significant effect in the 50-g. limestone intake.

The effects of limestone and dicalcium phosphate, fed in graded amounts alone and in combination, on the digestibility of the protein and the energy

TABLE 4

Effect of limestone on the digestibility of the protein and the energy of silages

(1950–51)

	No limestone[a]			100 g. Limestone/day[a]		
	Animal No.	Protein	Energy	Animal No.	Protein	Energy
		(%)			(%)	
Ladino-brome silage with molasses	16	57.52	60.64	11	53.32	53.81
	12	52.32	51.92	13	51.38	49.86
	15	55.72	57.73	14	52.92	52.48
	Av.	55.25	56.76	Av.	52.54	52.05
Timothy-SO_2 silage	12	48.19	58.37	11	46.55	57.03
	15	49.42	59.86	14	46.12	56.05
	Av.	48.80	59.11	Av.	46.33	56.54
Timothy-molasses silage	16	42.49	58.52	11	40.89	57.50
	15	40.37	55.60	14	39.39	54.95
	Av.	41.33	57.06	Av.	40.14	56.22
Ovér-all averages		48.46	57.64		46.34	54.94

[a] The differences between 0 and 100 g. limestone per day for both protein and energy were significant at the 1% level.

TABLE 5

Effect of limestone on the digestibilities of the protein and the energy of grass-legume silage preserved with molasses

(1951–52)

	No limestone[a]		50 g. limestone/day		100 g. limestone/day	
Animal No.	Energy	Protein	Energy	Protein	Energy	Protein
			(%)			
11	65.01	62.04	63.73	57.87	63.38	60.09
17	63.73	64.11	64.97	62.40	61.88	58.48
18	64.80	62.30	65.14	65.10	64.16	58.49
Av.	64.51	62.82	64.61	61.79	63.14	59.02

[a] Differences between 0 and 100 g. limestone for both protein and energy are significant at the 5% level.

of a mixed ration in the experiments of 1954–55, are shown (Table 6). Results of those experiments show that the 2% limestone decreased the digestibility of both protein and energy, thus confirming the previous work with silage. The 2% dicalcium phosphate did not have any appreciable effect. The addition of 2% dicalcium phosphate to the 2% limestone supplement minimized the depressing effect of the limestone. The 1% limestone supplement decreased the digestibility of the energy significantly, but its depressing effect on the protein of the ration was not significant. The 1% dicalcium phosphate did not affect the digestibility of the energy or the protein of the ration.

The data suggest that the addition of calcium to the ration depressed the digestibilities of both protein and energy; whereas, the addition of phosphorus increased the digestibility of the protein. The change in the calcium : phosphorus ratio does not explain entirely the results obtained, particularly with the mixed

TABLE 6

Effect of limestone and dicalcium phosphate on the digestibilities of the protein and the energy of a mixed dairy ration
(1954–55)

Animal No.	0-Minerals		2% limestone[a]		2% dicalcium phosphate[b]		2% limestone + 2% dicalcium phosphate		1% limestone[c]		1% dicalcium phosphate[d]	
	Energy	Protein	Energy	Protein	Energy	Protein	Energy	Protein	Energy	Protein	Energy	Protein
	(%)											
G 4	57.60	65.56	53.61	62.04	59.77	65.86	58.15	64.96	57.04	64.19	60.36	63.52
G 5	63.03	66.26	60.67	64.65	58.67	67.76	59.35	65.70	59.40	65.10	60.95	69.08
H 35	61.58	66.01	56.16	64.94	60.30	67.62	61.45	65.71	57.93	67.63	58.86	70.13
H 36	58.94	67.91	53.95	65.11	61.44	67.01	60.28	65.44	58.35	67.73	60.57	71.66
Av.	60.29	66.43	56.10	64.18	60.04	67.06	59.81	65.45	58.18	66.16	60.18	68.60

[a] Differences between 0 and 2% limestone are significant for both protein and energy.
[b] Differences between 0 and 2% dicalcium phosphate are not significant.
[c] Differences between 0 and 1% limestone in energy are significant at the 1% level, but the differences in the protein are not significant.
[d] Differences between 0 and 1% dicalcium phosphate for both energy and protein are not significant.

rations. It appears that the amounts of calcium and phosphorus also are factors. When the 2% limestone was added to the mixed ration, the digestibilities were depressed considerably. The addition of both 1 and 2% dicalcium phosphate tended to increase the digestibility of protein. When increased quantities of both calcium and phosphorus were added, the digestibility values were between these two extremes. There is nothing in these data which would incriminate the CO_3 radical for the decrease of digestibility, but this possibility should not be ignored. These results suggest the need for studies on the effect of calcium and phosphorus supplements, fed separately and in various ratios, on the digestibilities of protein and energy, as well as on feed consumption and growth.

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VALUE OF AN ORGANIC ARSENICAL IN THE RATION OF YOUNG DAIRY CALVES[1]

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SUMMARY

This study reports the results of supplementing the daily ration of the young dairy calf with 60 and 120 mg. of sodium arsanilate from the third day through the 8th wk. of age. Neither growth rate and efficiency of feed utilization, nor incidence of scouring, was significantly influenced by the presence of the supplement in the ration. No symptoms of arsenic toxicity were observed in any of the animals. These data indicate *(1)* that no beneficial effects can be expected from supplementing the ration of the young, healthy calf with sodium arsanilate and, *(2)* that the young calf is tolerant to high levels of this organic arsenical.

Inorganic arsenic has been used therapeutically in animals for well over 100 yr. *(7)*. The use of organic arsenicals in animal feeds has been reviewed by Frost *et al. (8)* and by Frost and Spruth *(9)*. Some evidence has accumulated which indicates that certain of the organic arsenicals have growth-promoting and/or disease-controlling properties for the young calf.

Graf and Holdaway *(11)* observed that calves fed arsanilic acid scoured less and made more rapid gains during the first 4 wk. of life than did animals not receiving the supplement. Slightly better results were obtained at levels of 60 and 120 g. per ton than when a 240-g. level was fed. Dewey *(5)* did not obtain any response from dairy calves supplemented with sodium arsanilate at levels of 20 or 40 mg. per 100 lb. body weight. However, some indication was obtained in the Texas work *(5)* that unthrifty calves might benefit from relatively small doses of sodium arsanilate or dodecylamine *p*-chlorophenylarsonate. In a study of the comparative value of several antibiotics and 3-nitro-4-hydroxy phenyl arsonic acid for young calves, Owen *et al. (17)* found that the arsenical gave results equal to or better than the antibiotics, in maintaining an increased rate of gain after the supplements were removed from the animals' rations.

Bartley *et al. (1)* supplemented the diets of calves from birth to 23 wk. of age with 50 mg. of arsanilic acid per calf daily. No statistically significant difference in weight gain or feed efficiency was observed between the supplemented and the control calves. Also, there was no apparent difference between the two groups in incidence of disease.

In an experiment designed to study the value of supplementing the calf's ration with an arsenical (arsanilic acid) plus an antibiotic (aureomycin) *(12)*, evidence was obtained which indicated that such a combination (60 mg. arsanilic acid and 25 mg. aureomycin per calf daily) was no more effective in increasing weight gains and decreasing the incidence of scours than either of the supplements fed alone at the same levels. The supplements, fed singly or in combination, did

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increase daily gains; however, the differences between the supplemented and the control calves were not significant, because of the large within-group variation. The animals used in this experiment were obtained from various state institutional herds in Virginia.

This study was undertaken *(a)* to obtain additional information on the value of an organic arsenical (sodium arsanilate) in the ration of the young calf and *(b)* to obtain information on the tolerance level of this arsenical for young dairy calves.

EXPERIMENTAL PROCEDURE

Twenty-four Holstein calves (12 males, 12 females) were used in this study. The calves remained with their dams for at least 24 hr. postpartum and subsequently were removed to individual pens and hand-fed colostrum twice daily for an additional 24-hr. period. On the third day postpartum, the calves were placed on the experiment and allotted at random to the following treatments: Group A (control)—basal; Group B—basal plus 60 mg. sodium arsanilate per calf daily, and Group C—basal plus 120 mg. sodium arsanilate per calf daily. The sex distribution was the same in each group.

The experimental treatments were imposed through the 8th wk. of age, during which time each calf received approximately 300 lb. of whole milk. All calves were fed a grain ration composed of the following poundages: ground corn, 600; ground oats, 580; wheat bran, 500; soybean oil meal, 280; iodized salt, 20, and steamed bonemeal, 20. Grain consumption was limited to a maximum of 4 lb. per day. Fair-quality mixed hay averaging 11% total protein was provided ad libitum.

The arsenical was dissolved in water and added to the milk at feeding time. Intake of the supplement approximated 0.007 to 0.013% of the total dry matter in the ration.

Feed consumption data were obtained on a daily basis for all calves. The calves were weighed and measured at the beginning and at the end of the experiment, also at weekly intervals between.

RESULTS

A summary of the growth data is presented (Table 1). The control calves and those receiving 120 mg. of arsenical daily made essentially the same body weight gains, whereas the 60 mg.-per-day group averaged somewhat lower. However, analysis of variance revealed that none of these differences in weight gain was great enough to be statistically significant.

Average increases in height of withers and in chest circumference were very similar in all groups. No significant differences with respect to these body measurements were observed between treatment groups.

The feed data (Table 2) suggest that both levels of the arsenical increased the consumption of hay, whereas the higher level also increased slightly the intake of concentrate. These differences in feed consumption were not significant, however. Statistical analysis revealed no significant differences in efficiency of feed utilization (calculated TDN per lb. weight gain) between treatment groups.

TABLE 1

Effect of arsenical supplementation on weight gains and certain body measurements
(eight calves per group)

Treatment groups[a]	Av. initial weight	Av. daily gain	Av. increase in	
			Wither height	Chest circumference
	(lb.)		*(in.)*	
A	89	0.94 ± 0.05[b]	2.7 ± 0.25	4.4 ± 0.35
B	87	0.87 ± 0.04	2.5 ± 0.24	4.4 ± 0.29
C	86	0.98 ± 0.02	2.5 ± 0.28	5.2 ± 0.34

[a] A = control, B = 60 mg. sodium arsanilate daily, and C = 120 mg. sodium arsanilate daily.
[b] Mean ± standard error.

TABLE 2

Effect of arsenical supplementation on feed consumption and efficiency of feed utilization
(eight calves per group)

Treatment[a]	Av. total consumption per calf				TDN per lb. gain
	Milk	Grain	Hay	TDN[b]	
			(lb.)		
A	305	57.1[c] ± 3.7	11.8 ± 1.6	96.0 ± 3.4	1.84 ±0.63
B	305	54.1 ± 2.6	14.1 ± 2.2	95.8 ± 1.8	2.03 ±0.44
C	305	62.1 ± 0.78	15.1 ± 0.60	101.9 ± 2.0	1.90 ±0.64

[a] A = control, B = 60 mg. sodium arsanilate daily, and C = 120 mg. sodium arsanilate daily.
[b] Calculated from Morrison's *(16)* tables.
[c] Mean ± standard error.

The incidence of scouring was very low in this experiment and was unrelated to treatment.

No clinical symptoms of arsenic toxicity were observed in any of the calves.

DISCUSSION

The data from this study do not support the preliminary findings of Graf and Holdaway *(11)* or the results of Owen *et al. (17)*; however, they do confirm the observations of Dewey *(5)*, as well as the data of Bartley *et al. (1)*. They are also in agreement with the more extensive studies of Graf *(10)*. The reason (or reasons) for these conflicting results is not apparent, but may be associated with the level and types of bacterial infection in the experimental animals. The form of the arsenical used also may be important. On the basis of the present investigation and the growth studies made by Dewey *(5)* sodium arsanilate, at the levels tested, would appear to be of little or no value in the feeding of calves free of any disease of major consequence. The work of Graf *(10)* and Bartley *et al. (1)* indicates the same to be true with respect to arsanilic acid. On the other hand, 3-nitro-4-hydroxy phenyl arsonic acid was reported to exert a clear-cut beneficial effect in the ration of young calves in the Iowa work *(17)*. Further work appears to be needed to establish the possible role and value of arsenicals in calf rations.

One of the major advantages found for the arsenical by the Iowa workers *(17)* was its apparent superiority in maintaining an increased growth rate subsequent to the removal of the supplement from the ration. The present study does not provide any information on this point; however, earlier observations *(13)* do not support this finding.

It might be theorized that the arsenical was ineffective in the present investigation, because diarrhea was not a problem even though the animals were housed in dirty quarters. It is of interest that in the study of Owen *et al. (17)* scouring was quite prevalent, and since none of the supplements fed was effective in controlling the diarrhea, it was proposed to be of noninfectious origin.

The fact that the arsonic acids are important as growth promotants and disease-control agents for swine and poultry *(8)* and of questionable value for young calves and older ruminants *(2, 3)* indicates that the rumen microorganisms might be implicated. With respect to the young calf, this explanation does not seem feasible because, in most of the studies reported, the animals used were essentially monogastric animals during the greater part of the experimental period when the supplement was fed. Also, in light of the recent work by Hegland *et al. (15)*, it seems doubtful that any large amount of the arsenical would enter the rumen when administered in the milk, especially if nipple-pails were used.

The results of the present study, as well as earlier work *(11, 12)*, indicate that both arsanilic acid and sodium arsanilate are tolerated at high levels in calves. In a limited investigation *(14)*, it was found that arsanilic acid fed with the grain ration was slightly toxic at the 0.04 to 0.05% level. There appears to be a rather wide margin of safety for certain of the arsenicals in fattening lambs *(4)* and beef cattle *(2, 6)*.

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EFFECT OF ZEOLITE TREATMENT ON HARD DRINKING WATER ON MILK PRODUCTION [1]

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SUMMARY

During November 1 to April 30 (181 days), periods of alternate years, 19 cows averaged 17.6 lb. of milk daily when receiving untreated well-water averaging 190 p.p.m. of total hardness, as compared to 19.9 lb. when receiving water from the same source but zeolite-treated to zero-hardness. The average stages of lactation (after calving) were 169 and 132 days, respectively. When adjusted for stage of lactation by covariance analysis, the corrected yields were 18.8 and 19.0 lb. for untreated and treated water, respectively. This difference is not significant. It is concluded that softening the drinking-water for dairy cows has no measurable effect on milk production. When given a choice between untreated and treated water during an entire summer, the herd showed no clear-cut preference for either, although they drank more of the untreated water.

Water is the nutrient required in greatest quantity by dairy cows, and it plays a critical part in their nutrition. The water for many dairy herds is supplied by deep wells and contains considerable amounts of minerals, with calcium and magnesium compounds usually the most prominent. Henry *(3)* compared rain-water with hard well-water for pigs, with the thought that the calcium of the hard water might be beneficial. However, there was no evidence of any effect, favorable or unfavorable. During recent years, there has been some interest in the possible value of softening the drinking-water of dairy cattle. Graf and Holdaway *(2)* found no difference in milk production or gain in weight due to zeolite treatment of well-water of about 290 p.p.m. total hardness, when supplied to milking cows during 57-day periods. Water consumption, and ratio of milk produced to water consumed, were not affected by treatment of the water. Blosser and Soni *(1)* carried out a similar experiment with 27 cows over 6-wk. periods, with no significant difference in milk production, water consumption, or water : milk ratio. The work reported in this paper was planned to test the effect over longer periods of time.

EXPERIMENTAL PROCEDURE

The entire milking herd of 24 Guernsey cows at the LaCrosse station of the University of Wisconsin was divided into two groups, balanced as nearly as possible as to age and stage of lactation. The water bowls of half of the stalls were connected to a line from a deep well, supplying water averaging 190 p.p.m. total hardness. The remaining bowls were connected to a line supplying water from

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the same source, but treated by a zeolite process to zero-hardness. Both lines were metered, to give an accurate measure of total water consumption. The meters were read daily. The softening units were of a tank-replacement type, changed regularly to insure water of zero-hardness at all times.

Daily milk weights were recorded, and fat was determined on monthly samples.

The experiment on the cows was started on November 1 and continued through April 30. During the first winter season, Group A received the zeolite-softened water, and Group B the untreated. During the following pasture season, all cows had access to tanks containing softened water. For the second barn-feeding period the two groups were reversed, Group A getting the untreated and Group B the zeolite-treated water.

The results for the two 6-mo. periods are shown (Table 1).

TABLE 1

Relation of hardness of water to milk production

	Hard water			Soft water		
Cow No.	Days milked	Av. days fresh	Milk *(lb.)*	Days milked	Av. days fresh	Milk *(lb.)*
	Group A			Group A		
112	110	254	896	136	203	1,387
170	149	75	3,259	142	71	3,313
268	181	330	1,598	105	170	2,652
248	124	220	1,032	158	227	1,627
271	181	184	2,198	182	176	3,071
262	132	168	2,184	134	122	2,842
306	130	205	3,122	141	92	2,845
266	177	89	3,689	182	91	3,512
276	107	172	2,302	113	57	2,454
288	119	158	2,616	60	30	1,515
	Group B			Group B		
105	181	95	3,984	182	113	3,792
277	181	163	4,437	182	102	4,310
241	181	270	1,317	128	246	2,654
215	181	125	4,244	182	120	3,679
272	181	184	3,092	182	176	3,532
307	100	75	2,399	137	157	2,455
263	144	72	4,131	140	78	4,543
285	130	144	1,843	123	67	2,222
165	123	232	1,261	94	206	1,419
Av.	148.0	169.2	(daily) 17.6[a]	142.3	131.8	(daily) 19.9[b]

[a] Adjusted for stage of lactation, (daily) 18.8.
[b] Adjusted for stage of lactation, (daily) 19.0.

Because all the milking cows of the herd were used, and the experiment covered a 2-yr. period, it was inevitable that there should be some change in the make-up of the herd. Nineteen cows were in the herd through the entire experiment, and seven were not. These seven are omitted from Table 1, which includes the data on all cows which had a full period on each treatment.

The reversal design of the experiment equalized age of cow between treatments, and to a large extent the differences between the two seasons. However, since the records cover a definite calendar span, the days in milk and the stage of lactation were not equal.

DISCUSSION

Nineteen cows were in the herd during both periods of the experiment. Ten had treated water during the first period, and nine during the second. Each group received the untreated well-water during the alternate periods. These 19 cows averaged 19.9 lb. of milk per day during 142 days in milk on treated water, as compared to 17.6 lb. for 148 days in milk on hard water. During the soft-water periods, the average stage of lactation (average days after calving) was 132 days, as compared to 169 on hard water. The apparent average daily milk production advantage for the treated-water periods was 2.3 lb. However, there was an advantage of 37 days for earlier average stage of lactation.

In an individual lactation, a period of 181 days averaging 132 days post-partum would include the span of from 42 to 222 days, inclusive; whereas, an average stage of lactation of 169 days would take in the span of 79–259 days, inclusive. Since the 79- to 222-day portion is common to both, the earlier stage of lactation would enhance production to the extent that the 42–78 days' yield exceeds the 223- to 259-day production. A comparison of corresponding spans within lactations of these cows during the course of the experiment gave an advantage of 2.8 lb. in average daily milk yield for the earlier period. This suggests that the apparent advantage of the treated water may have been entirely owing to the earlier average stage of lactation.

The data were subjected to covariance analysis, to test this observed difference. The average daily milk yields, adjusted by this treatment for stage of lactation, were 19.0 and 18.8 lb. for treated and untreated water, respectively. The difference of 0.2 lb. is not significant. Therefore, it may be concluded that with the type of well-water supplied to the cows in this experiment, averaging about 190 p.p.m. of total hardness, softening by zeolite had no appreciable influence on milk production. These results over 181-day periods are in agreement with those reported by Graf and Holdaway *(2)* for 57-day periods and by Blosser and Soni *(1)* for 6-wk. periods.

Average daily water consumption for 24 cows differed by less than 1 lb. Because the water was metered by groups and some cows, due to unavoidable herd turnover, were not in the experiment through the entire 2-yr. span, it was not possible to arrive at accurate figures for comparing water consumption per pound of milk produced. If we assume that the 19 cows which were compared for milk production drank a pro-rata share of the total water, the consumption was 3.9 lb. of untreated against 3.4 lb. of treated water per pound of milk produced.

In order to check for possible preference, two similar float-equipped tanks were placed in the barn-lot during the second summer of the experiment. One was supplied by the soft-water line, the other by the hard. Both were metered. The entire herd had free choice between the two types of water, with access to no other. During the 103-day period, they drank 11,230 gal. (56% of total) of untreated and 8,810 gal. (44% of total) of treated water. These observations do not indicate any serious discrimination against either.

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TECHNICAL NOTES

EFFECT OF FEEDING VARIOUS LEVELS OF 2,6-DI-TERTIARYBUTYL-4-METHYL PHENOL TO LACTATING DAIRY COWS [1]

The antioxidant 2,6-di-tertiarybutyl-4-methyl phenol (BHT) has been approved as an additive to poultry rations and also has been approved for human consumption *(1)*. Although a recent report by DeLuca *et al. (2)* indicated that BHT, when fed at 0.01% of ration (90% dry matter basis), was ineffective in reducing the incidence of copper-induced oxidized milk flavor, the possible effectiveness when higher levels of BHT are fed to lactating dairy cows was recognized and the present study was undertaken to explore this possibility.

Two separate trials were conducted, Experiment I from February to March, 1956, with 16 cows (12 Guernseys and four Holsteins) and Experiment II from November to December, 1956, also with 16 cows (eight Guernseys and eight Holsteins). The animals were selected for producing milk which would exhibit oxidized flavor 72 hr. after the addition of 5.0 p.p.m. of Cu^{++}. The cows were randomly assigned in replicates of four animals each, restricted with respect to breed, to one of four levels of BHT—0.00, 0.01, 0.03, or 0.09% of ration (90% dry matter basis). The BHT supplement [2] was premixed with linseed oil meal, so that 4 g. of this premix would be sufficient for 1.0 lb. of ration, and was mixed into the P.M. grain allowance each day of the feeding period.

The experiments consisted of a 1-wk. standardizing period during which only the basal ration, consisting of grass silage, alfalfa hay, and grain, was fed, and a 4-wk. comparison period, during which BHT was included at the indicated levels. The ration allowance was held constant throughout the experiments. Twice weekly during the entire experimental period, milk samples of the P.M. milking were obtained for organoleptic testing. The samples were tested 72 hr. after milking, independently by two judges, without and with the addition of 1.0 and 5.0 p.p.m. of Cu^{++} from copper sulfate. Once weekly, milk weights were recorded and samples of the A.M. and P.M. milking were composited for butterfat analysis. Milk samples obtained at the termination of Experiment I were assayed for BHT content *(3)*.[3] Statistical procedures as outlined by Snedecor *(4)* were employed in the analysis of the data.

No statistically significant differences were observed in the milk production criteria, either between levels of BHT fed or between the BHT-supplemented groups and the negative controls. The following averages for the comparison period, with their standard deviations, were observed for Experiment I and Experiment II, respectively: lb. of milk, 33.0 ± 10.0 and 28.1 ± 12.6; % butterfat, 5.0 ± 0.6 and 4.7 ± 1.5, and lb. 4% FCM, 37.0 ± 12.0 and 29.6 ± 15.1. Whereas covariance, utilizing initial production and/or days in milk, reduced the size of the experimental error, the nonsignificance of the treatment differences was not altered.

Results of the organoleptic tests are presented (Table 1), expressed as per cent samples free from oxidized flavor. No statistically significant reduction in the incidence of copper-induced oxidized flavor was noted for the BHT-supplemented groups *versus* the negative controls, or between levels of BHT fed when 1.0 p.p.m. Cu^{++} had been added to the milk. This may have been owing to the cows' resistance to the induction of oxidized flavor when this lower level of copper was employed. Considering both experiments, there was a slight progressive reduction in the incidence of oxidized flavor occurrence with increasing levels of BHT fed, with complete inhibition when 0.09% BHT had been fed. In the presence of 5.0 p.p.m. Cu^{++}, a significant reduction in oxidized flavor was observed for the group fed 0.09% BHT and a slight, nonsignificant reduction for the feeding of 0.03%. As previously reported *(2)*, the 0.01% level appeared ineffective in reducing the incidence of copper-induced oxidized milk flavor.

Estimates of the minimum level of BHT to feed to prevent copper-induced oxidized milk flavor were based on regressions of per cent samples free from oxidized flavor on level of BHT fed. The estimates were as follows for Experiment I and Experiment II, respectively: 1.0 p.p.m. of Cu^{++}, 0.06% and 0.08% BHT; 5.0 p.p.m. Cu^{++}, 0.19% and 0.18% BHT of ration (90% dry matter basis). These estimates may be too great for application to field conditions, and no data are available for cows whose milk becomes oxidized spontaneously.

Although some interference was evident in

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[2] The BHT supplement, containing approximately 50% BHT and 50% calcium chloride, was supplied through the courtesy of R. F. Elliott, American Cyanamid Co., Pearl River, N. Y.

[3] Mrs. M. Booth of the American Cyanamid Co., Pearl River, N. Y., conducted the BHT analyses of the milk.

TABLE 1

Effect of level of BHT on incidence of oxidized milk flavor

	Per cent of BHT added to ration				Standard deviation of a cow
	0.00	0.01	0.03	0.09	
Samples free from oxidized flavor			*(%)*		
Fresh					
Experiment I					
Standardizing	100.00[a]	93.75	100.00	100.00	6.25
Comparison	98.33[b]	100.00	100.00	100.00	1.67
Experiment II					
Standardizing	100.00	100.00	100.00	100.00	0.00
Comparison	100.00	100.00	100.00	100.00	0.00
1.0 p.p.m. Cu^{++}					
Experiment I					
Standardizing	68.75	68.75	62.50	62.50	35.59
Comparison	95.00	81.66	98.33	100.00	18.73
Experiment II					
Standardizing	75.00	68.75	62.50	62.50	32.87
Comparison	54.69	73.44	93.75	100.00	28.28
5.0 p.p.m. Cu^{++}					
Experiment I					
Standardizing	0.00	6.25	0.00	6.25	7.22
Comparison	16.67	10.00	21.67	54.74	20.13
Experiment II					
Standardizing	0.00	0.00	0.00	0.00	0.00
Comparison	0.00	0.00	7.81	50.00	15.81
BHT fed			*(g/cow/day)*		
Experiment I	0.00	2.0	5.7	17.4	.....
Experiment II	0.00	2.0	6.4	18.1	.....
Concentration of BHT in milk			*(γ/100 ml.)*		
Experiment I	8	18	52	72	36

[a] Values observed before feeding BHT.
[b] Mean of 15 observations during the feeding of BHT.

the BHT assay, as indicated by a positive response with the controls (Table 1), BHT apparently was transferred to the milk in increasing amounts with the level fed.

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EFFECT OF *PARA*-AMINO SALICYLIC ACID ON GROWTH OF MALE HOLSTEIN CALVES [1,2]

It has been suggested that *para*-amino salicylic acid (PASA) would stimulate the growth of calves when included in the diet *(2)*. A feeding trial has been conducted to test further this possibility.

Twelve male Holstein calves were divided into two groups at random. All calves had received colostrum for at least one day before being placed on trial. They were fed limited amounts of whole milk until ten days of age, along with milk replacement which was started at six days of age and continued through 42 days of age. Each calf received a total of 60 lb. of milk and 39.4 lb. of dry milk replacement. Calf starter was fed ad libitum until a maximum of 4 lb. was being consumed by each calf daily. No antibiotic was included either in the milk replacement or in the starter. The roughage used was second-cutting mixed alfalfa-grass hay and was fed ad libitum. The diet fed the experimental group (Group I) was similar to that fed the control group (Group II), differing only in that it contained PASA at the rate of 90 mg/lb of milk replacement and 50 mg/lb of starter. The experimental animals consumed PASA at a rate of approximately 90 mg/100 lb. of body weight per day. Management and environment were the same for both groups.

Results of the trial (Table 1) show that Group II gained more in body weight, height at withers, and chest circumference than did Group I. The differences in favor of Group II were statistically significant at the 1% level of probability for body weight and at the 5% level for height at withers and chest circumference. Group II also required fewer pounds of TDN per pound of gain than did Group I, indicating a decrease in efficiency of utilization of TDN in the experimental group. These results suggest that PASA, when fed at the rate of approximately 90 mg/100 lb. body weight per day and without antibiotics, was not an effective growth stimulant for Holstein calves.

TABLE 1

Growth performances of calves fed para-*amino salicylic acid as compared to controls* [a]

	Group I (Fed PASA)			Group II (Controls)		
	Initial	6 wk.	12 wk.	Initial	6 wk.	12 wk.
Body weight *(lb.)*	101	136	191	104	144	214
Height at withers *(cm.)*	75.0	80.2	84.9	75.2	80.7	86.7
Chest circumference *(in.)*	30.8	33.9	37.6	31.3	34.7	39.0
TDN/lb gain			2.00			1.84

[a] Data represent means of six calves per group.

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[1] Authorized for publication on September 24, 1957, as Paper No. 2200 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] The *para*-amino salicylic acid used in this work was supplied by Chemo Puro Manufacturing Corp., Newark, N. J.

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TOXICITY OF NITRATES AND NITRITES TO DAIRY CATTLE

During the past decade, the use of nitrogen fertilizers in the production of forage crops has become a wide-spread practice in many areas of the tropics. This practice has led to tremendous increases in yields of total digestible nutrients per acre. In tropical areas with 70–80 in. of rainfall annually, the yields of dry forage per acre can be increased from 4–5 tons per year to 25–30 tons per year. This increase in over-all yield is accompanied by a threefold increase in

the per cent crude protein, resulting in an 18- to 20-fold increase in total crude protein per acre. These yields are phenomenal when compared to forage yields obtained in the temperate zones.

The utilization of these high protein grasses has presented problems. These grasses have a relatively high nonprotein nitrogen fraction, including nitrates, which leads to undesirable effects unless certain specified feeding practices are adopted *(2, 5)*.

Recently, very severe losses from toxic agents of unknown origin occurred at two dairy farms in Puerto Rico. One dairyman lost 23 milk cows from his herd of 60; another lost 28 heifers. In both cases the losses occurred within 48 hr. after the first symptoms. These losses were not fully explained and, as both dairies were utilizing high-nitrogen forage, the possibility that nitrates were the causative agent was investigated. This investigation included four feeding trials. This report describes these trials and presents the results.

The grass, *Pennisetum purpureum*, used in these trials was produced on ¼-, ½-, and ¾-acre field plots. A high nitrate content was obtained by the application of 400 lb. per acre of a 10-10-10 fertilizer one week after cutting, followed 4 wk. later by an application of 800, 1,600, or 2,000 lb. of ammonium sulfate per acre. The grass was harvested and fed when 6 to 8 wk. old, depending on the nitrate concentration desired. Each individual ration was prepared daily by weighing both the fresh, finely chopped grass and the concentrate. All animals received the principal nutrients in excess of their requirements *(3)*. Feces and urine samples were collected manually. Nitrate concentrations in all samples were determined by the method of Swann and Adams *(4)*, and the methemoglobin levels by the procedure of Evelyn and Mallory *(1)*.

Trial 1. Two pregnant, nonlactating dairy cows, weighing about 950 lb. each, were placed in feeding pens and maintained on a chopped-grass ration that was free of nitrates. No supplements were given. An excess of fresh grass was available in the feed boxes at all times. After 2 wk., the nitrate-free grass was gradually replaced over a period of 1 wk. by a grass that contained 0.3 to 0.4% potassium nitrate (KNO_3) on a fresh weight basis (1.3 to 1.8% dry weight basis). The high-nitrate ration was then fed continuously for 60 days. The daily consumption of one cow varied from 115 to 175 lb., and averaged 135 lb.; that of the other from 125 to 210 lb., averaging 155 lb/day.

Weekly determinations of methemoglobin showed that the levels varied between 2 and 7%, and averaged 5%. Periodic inspections by a veterinarian revealed no symptoms of toxicity. One animal refused to eat on the 34th day but was normal again the next day. Thirty days after the feeding trial terminated one cow gave birth to a normal calf. The other cow gave birth to a normal calf 45 days after the trial ended.

Trial 2. A dairy herd of eight lactating and four nonlactating cows was switched from a nitrate-free pasture roughage to a high-nitrate, chopped roughage (1.8% potassium nitrate on a dry weight basis). The chopped roughage was fed from 12, 3 by 2.5 by 1.5 foot feeding boxes located in a large feeding pen. An excess of chopped grass was maintained in the feeding boxes at all times. Concentrate was fed at milking time in recommended quantities *(3)*. Feeding of the high-nitrate roughage continued 4 wk. The average consumption of roughage was 87 lb/cow/day over the 4-wk. period (336 cow-days).

Blood samples taken at 8 A.M. on the 22nd day of the trial contained normal amounts of methemoglobin, i.e., 3 to 5%. A veterinarian found the herd normal at the end of the trial. Milk production records revealed a slight decline not associated with the high nitrate content of the roughage.

Trial 3. Two yearling bulls, weighing approximately 400 lb. each, were placed on a balanced ration made up of a low-nitrogen roughage and a high-protein concentrate. After 1 wk., the animals were treated for parasites, and the low-nitrogen roughage was replaced with equal amounts of chopped grass which contained from 0.3 to 0.4% potassium nitrate (1.3 to 1.7% dry weight). This ration was fed for 2 wk. and the methemoglobin remained at normal levels. At the end of this 2-wk. period, the high-nitrate forage was replaced with a forage free of nitrates. Chemically pure potassium nitrate was then added to the forage until it contained 1.7% on a dry weight basis. This required 120 g. of potassium nitrate for the daily ration of each animal. After 2 wk., both animals appeared normal and continued to gain weight. The concentration of nitrates and nitrites in the feces and urine showed that less than 4% of the dietary nitrates was being excreted in the form of nitrates and nitrites. The concentration of potassium nitrate in the roughage was then raised from 1.7 to 3.0%. This required 210 g. of potassium nitrate for the daily ration of each animal. The animals were then maintained on this ration for 30 days, and together they consumed almost 30 lb. of potassium nitrate.

During this period, only 1% of the dietary nitrates was recovered unchanged in the feces, and approximately 3% passed unchanged in the urine. Methemoglobin levels ranged from 5 to 8%. At the end of the trial, the animals were examined by a veterinarian and appeared normal.

Trial 4. The two animals in Trial 3 were switched directly to Trial 4. The potassium nitrate (210 g.) in each daily ration was replaced by an equimolecular quantity of potassium nitrite (177 g.). After 1 wk., the methemoglobin levels were 3 and 7%. The quantity of potassium nitrite in each daily ration was raised to 200 g. Two weeks later the animals appeared normal to the examining veterinarian and the

trial was terminated. Methemoglobin levels were 2 and 3% at the end of the trial.

These trials led the authors to the conclusion that the severe losses mentioned previously were not caused by the nitrates in the forage being fed. This conclusion is based on these facts: *(1)* The losses occurred in herds that were obtaining 75% of their requirements from a forage that contained no more than 1.0% potassium nitrate on a dry weight basis. *(2)* Test animals showed no symptoms of nitrate poisoning and continued to gain weight while being fed rations that contained 3.0% potassium nitrate.

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OUR INDUSTRY TODAY

NEW DEVELOPMENTS IN THE PROPAGATION OF LACTIC CULTURES: CULTURE MEDIA AND BACTERIOPHAGE INHIBITION [1, 2]

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Lactic cultures which do not produce acid uniformly every day present an important problem to the dairy industry. Studies on the causes of slow acid production, or complete failure of a culture to produce acid, indicate that bacteriophage often is responsible. Since bacteriophage has been recognized as an important cause of culture failure, many recommendations have been made to limit its entrance into cultures and to decrease its activity. Culture selection, sanitation programs, culture rotation, and propagation of mother cultures and preparation of intermediate and bulk cultures in a relatively bacteriophage-free area, have been helpful in decreasing culture failures. However, these methods have not entirely eliminated the bacteriophage problem.

A medium which would support good growth of lactic cultures, but which would not permit bacteriophage development would be of value to the dairy industry, provided that the use of such a medium would not constitute adulteration. The addition to a medium such as milk, of some nontoxic substance that would prevent bacteriophage development but permit good growth of the culture organisms, would also have possibilities. In 1949, Shew *(5)* and in 1956, Reiter *(3)* reported that bacteriophage active against the lactic streptococci require calcium for maximum development. Potter and Nelson *(2)* and Doull and Meanwell *(1)* developed media which were deficient in calcium and which supported growth of lactic cultures. These media were used for fundamental studies on bacteriophage. Reiter *(4)* described a medium, based on milk, in which bacteriophage did not multiply because of a lack of calcium, but in which certain lactic cultures grew normally. The medium was designated PRM (phage-resistant medium).

EXPERIMENTS WITH PRM

A supply of PRM was made available for experimental purposes by Fisons Milk Products Ltd., Loughborough, Leicestershire, England. The material was received in dry form and was reconstituted at the rate of 90 g. powder to 910 ml. of distilled water. After reconstitution, it was heated in flowing steam for 1 hr.

The first series of experiments with PRM were designed to determine whether cell lysis would occur in the medium. Two flasks containing skimmilk and two flasks containing PRM were inoculated with 1% lactic culture (LC-4). One flask of skimmilk and one flask of PRM also were inoculated with 1% of a bacteriophage preparation (B-14) active against Culture LC-14 and containing 10^5 particles per milliliter. The flasks were incubated at 70° F. The flask of skimmilk containing culture only, and both flasks of PRM, were coagulated in 16 hr. The flask containing skimmilk, culture, and bacteriophage was not coagulated in 24 hr. Bacteria-free filtrates were prepared from the material in each flask and the filtrate tested for the amount of bacteriophage present. The following results were obtained:

Media	Bacteriophage concentration
	(particles/ml)
Skimmilk + culture	< 1
Skimmilk + culture + bacteriophage	10^9
PRM + culture	< 1
PRM + culture + bacteriophage	< 1

Another trial was conducted in the same manner, except that Lactic Culture LC-3 and Bacteriophage Preparation B-3 were used. The bacteriophage preparation contained 10^9 particles per milliliter. As in the previous trial, the flask containing skimmilk and culture only, and both flasks of PRM, were coagulated in 16 hr. The flask containing skimmilk, culture, and bacteriophage was not coagulated in 24 hr. Bacteria-free filtrates were prepared and filtrates tested for the amount of bacteriophage present. The following results were obtained:

Media	Bacteriophage concentration
	(particles/ml)
Skimmilk + culture	< 1
Skimmilk + culture + bacteriophage	10^{11}
PRM + culture	< 1
PRM + culture + bacteriophage	< 1

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[2] Presented at Research Conference held at Beltsville, Maryland, October 30, 1957, by the Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

The data obtained in these, and in additional trials, indicate that bacteriophage does not multiply in PRM. One would assume that some bacteriophage would be detected in a filtrate prepared from a culture grown in PRM and to which many bacteriophage particles were added. However, it is known that bacterial cells will adsorb bacteriophage in the absence of calcium, and that the cells plus the adsorbed bacteriophage would be removed in the preparation of the filtrate.

Experiments also were conducted to determine the ability of PRM to support growth of the organisms in lactic cultures. In these experiments PRM was reconstituted at the 10, 9, and 7.5% levels. The reconstituted material was heated in flowing steam for 1 hr. before use. When cultures regularly propagated in skimmilk (9% solids) were transferred to both skimmilk and PRM, the coagulation times were very nearly the same, regardless of the level at which PRM was reconstituted. However, when tfansfers were made from PRM to PRM, the coagulation times generally were greater than those of the same cultures transferred from skimmilk to skimmilk, or from PRM to skimmilk.

Six cultures which had been prepagated regularly in skimmilk were propagated in both skimmilk and PRM (9% solids) for three propagations. Then, both sets of cultures were tested for rate of acid production in milk which had been heated for 1 hr. in flowing steam and cooled to 86° F. The milk was inoculated with 1% of each culture, incubated at 86° F. for 6 hr. and the amount of acid determined by titration. The acidities, calculated as per cent lactic acid, were as follows:

Culture No.	Transfer from skimmilk	Transfer from PRM
	(%)	
4	0.62	0.46
5	0.57	0.41
6	0.60	0.36
12	0.50	0.29
14	0.57	0.42
18	0.70	0.44

These results indicate that cultures grown in PRM do not produce acid as rapidly as cultures grown in skimmilk. Additional trials, in which plate counts were used to determine the rate of growth of lactic cultures in skimmilk and PRM, showed that skimmilk was the more favorable growth medium. The plate counts showed variations among cultures with respect to growth in PRM; the medium was more suitable for some cultures than for others. However, in no instance did the growth rate in PRM exceed the growth rate of the same culture in skimmilk under the same conditions.

Several cultures were available which coagulated milk in a normal manner but which contained bacteriophage in rather low concentration. Bacteria-free filtrates from four such cultures showed from 10^3 to 10^4 bacteriophage particles per milliliter. Because some previous experiments had indicated that it is rather difficult to eliminate bacteriophage from such cultures, it was of interest to determine whether cultures could be freed of bacteriophage by propagating them in PRM. Bacteria-free filtrates from the first propagation in PRM showed no bacteriophage in 1 ml. of filtrate from three of the cultures; one culture yielded a filtrate containing about 50 particles per milliliter. Filtrates prepared from the second propagation in PRM showed no bacteriophage in 1 ml. of filtrate from any of the cultures.

The experiments conducted with PRM indicate that:

Bacteriophage active against the lactic streptococci of dairy cultures does not multiply or cause cellular lysis in PRM when the product is reconstituted with distilled water.

A bacteriophage-carrying culture may be freed of bacteriophage by propagation in PRM.

Lactic cultures do not grow in PRM as well as in skimmilk.

Lactic cultures propagated in skimmilk produce acid more rapidly when transferred to milk than cultures propagated in PRM and transferred to milk.

Lactic cultures show variations with respect to their ability to grow in PRM; the medium is more suitable for some cultures than for others.

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UTILIZATION OF WHEY SOLIDS IN FOOD PRODUCTS[1]

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Whey solids have been consumed in many food products during the last few years. To convert this perishable material into a high-quality food requires careful selection of raw milk and the processing and refrigerating of whey and concentrated whey under controlled conditions. Dumping fluid whey into sewage disposal systems is expensive, and it is unlawful in many states to put whey and dairy wastes into streams and rivers.

The cheese industry, in one respect, is no different from other industries. All industries, to prosper in competitive markets, must constantly change their utilization of by-products, as well their ways of producing and marketing them.

Great strides have been made in utilizing whey solids, a by-product of the cheese industry. A large investment in equipment, and a real concern for high quality, are necessary profitably to produce whey solids that will be accepted by the food industry. Centralized cheese-manufacturing operations with modern processing and drying plants have made it possible to make an economical whey product from milk of good quality.

The quality specifications for whey solids of U. S. Extra Grade, effective July 8, 1954, depend upon the initial processing of the product and the nature of the cheese operation. The following steps are involved in processing whey from good-quality milk:

1. The whey is pumped from the cheese vat to a holding tank and then to a centrifugal separator, where the fat is removed as whey cream. U. S. Extra Grade whey solids must contain not more than 1.25% fat.
2. The whey is next pumped to holding tanks which are connected in series, to maintain a constant flow to the evaporators and to enable the operator to test the quality of the whey from different sources.
3. The whey is next preheated to 220° F. and pumped into three- or four-effect evaporators, where it is concentrated in stages. The temperature in the first stage is 200° F., which is decreased to about 140° F. in the last stage. Thus, whey containing 6% solids is condensed to about 40 to 70% solids.
4. The concentrated whey is cooled to about 35° F., pumped into tanks, and transferred to the final drier. To maintain the highest quality standards, this concentrate is hauled not to exceed 40 miles, in stainless tanks, to be dried.
5. It is necessary to stabilize the lactose in the whey concentrate to the monohydrate stage during the drying process, because the anhydrously dried product is very hygroscopic.

The concentrated whey may be dried by tunnel- or shelf-drying, drum- or roller drying, or by spray-drying.

In tunnel-drying, the concentrated whey, containing 70% solids, flows from the vacuum pan into a vat, where it is seeded with a small amount of dried whey from a previous batch and held not more than 24 hr. to allow the lactose to crystallize. Then it is spread on 1/16- to 1/12-mesh screen trays and dried in a tunnel where air circulates at from 140 to 180° F. At the higher temperatures, drying may be complete in from 3 to 4 hr. The dried whey is relatively nonhygroscopic. There are several variations of the tunnel-drying process.

In the drum-drying process, the whey is concentrated to 50% solids and flows to a series of drum driers. The rolls of each unit are 1/16-in. apart and operate at about 300° F. The whey dries in light-brown sheets which are ground and packaged. The resulting product varies in its hygroscopic property. There are several variations of this process.

Spray-drying of concentrated whey is accomplished with equipment used to dry milk. The lactose should be crystallized before drying; otherwise, a very hygroscopic product will result. The whey is preheated to 180° F. for 20 min., then concentrated to 40% solids. This concentrate is sprayed into the drying chamber at 285° F. The finished product is amorphous and somewhat hygroscopic. The whey proteins are soluble. There are also variations of the spray-drying process.

The following figures represent the average composition of fluid whey and whey solids:

	Water	Fat	Lactose	Whey proteins	Minerals
	(%)				
Fluid whey	94.0	Trace	4.5	0.8	0.7
Whey solids	4.0	1.0	71.0	13.0	11.0

Lactose constitutes about 40% of the total solids of milk. It is a reducing sugar of relatively low sweetness. It has a sweetness value of 16, compared to 100 for sucrose. Lactose is more resistant to acids than sucrose; therefore, is not as easily hydrolyzed or inverted. For this rea-

[1] Presented at Research Conference held at Beltsville, Maryland, October 30, 1957, by the Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

son, lactose is absorbed from the intestine more slowly than sucrose. The solubility of lactose is much less than sucrose.

The principal proteins in whey are lactalbumin and lactoglobulin. Commercial lactalbumin is a product which is precipitated by heating acidified whey and includes both the lactalbumin and lactoglobulin fractions. The whey proteins are heat-coagulable, depending on the intensity of heating when milk is processed for the various products. The whey proteins are not coagulated by the enzyme, rennin, or by dilute acids; consequently, they are always found in the whey. They have a high nutritional value.

The whey proteins have definite advantages in soups and in sterile creamed soups because they impart emulsion stability, thus greatly retarding the separation of discrete particles and of colloidal phases. In certain types of soups, where the color and acidity are such that regular milk products can not be used, the use of whey solids will not mask the desired color and will produce a smooth, tender body, also.

The baking industry, the largest single potential user of edible-grade dairy solids, has found whey solids to be functionally and economically basic for bakery products. Whey solids can be used as an additional ingredient, as a blend with other dairy ingredients, and as the complete dairy solids in a formula. They are being used for bread, rolls, fermented sweet products, cakes, cookies, crackers, pie crusts, biscuits, doughnuts, icing, and fillings. The loaves containing whey solids showed advantageously, having desirable crust color, color of crumb, softness, resilience, and shelf-life. Owing to the high percentage of lactose, whey solids can be substituted for dextrose and sucrose in many baking formulas.

In baking, owing to the fact that the whey proteins are not readily affected by the low pH of fermented doughs, they will add to the extensibility of doughs during the initial processing period. Because they are heat-coagulable, they will also contribute to structure during baking.

In ice cream, the use of whey solids at a level of 25% of the total serum solids produces a product with improved body, excellent flavor, and texture. The improvement in the body of the ice cream is attributed to the superior emulsifying and water-holding properties of the whey proteins.

In soft-served ice cream, whey solids can replace about 50% of the solids-not-fat. In sherbets, they are used at the 100% level, thus improving flavor, protein stability, and palatability.

In confections such as fudge, caramel, and coatings, whey solids accentuate added flavors and also contribute a rich, creamy flavor. Owing to the low caramelization point of lactose, whey solids will develop a brown color. Lactose caramelizes at 265° F., as compared with 310° F. for dextrose and 325° F. for sucrose. Whey also extends the shelf-life of candies. Lactose retards crystallization, thus controls graining-out. It is recommended that whey solids be added in various replacement levels, in initial tests to determine optimum levels.

In cheese spreads and cheese foods, whey solids are used effectively to provide a more desirable consistency to the product and to carry flavor.

Including whey solids in comminuted meat products and meat loaves of various types results in better color, moisture retention, less shrinkage, and improved palatability. Their use has recently been approved by the Meat Inspection Division of the USDA.

Food manufacturers are interested in whey solids and research is contributing new uses for this important dairy product.

ABSTRACTS OF PAPERS TO BE PRESENTED AT THE FIFTY-THIRD ANNUAL MEETING of the AMERICAN DAIRY SCIENCE ASSOCIATION

North Carolina State College
Raleigh
June 16–19, 1958

MANUFACTURING SECTION

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M1. Density of milk at low temperatures.* P. D. WATSON, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The densities of a variety of herd milks at low temperatures were determined precisely to provide data to establish volume-weight relationships more accurately than is possible from the literature. Specially designed pycnometers with a capacity of 46 ml., and refrigerated baths controlled to within ±0.02° C., were used. Duplicate determinations were made at two temperatures (0.95 and 4.95, or 0.95 and 9.85° C.) on 105 milk samples from representative sources. The percentage of milk fat in most samples was determined by the Babcock method, and in some of them by the gravimetric method. The percentage of total solids was measured gravimetrically. The percentages of fat, total solids and nonfat solids covered the following ranges, respectively: 3.1 to 6.4; 11.6 to 16.1, and 8. 2 to 10.2. The range of densities at 0.95, 4.95, and 9.85° C. were, respectively: 1.0313 to 1.0387; 1.0326 to 1.0374, and 1.0298 to 1.0369. Tables relating the density to the fat and solids-not-fat will be presented. The results emphasize that density is more closely correlated to the percentage of nonfat solids than to the percentage of fat in the milk.

*This work was financed by the Agricultural Marketing Service.

M2. Interrelation between conductivity, per cent lactose, and freezing point of milk. I. I. PETERS AND R. R. SHRODE, Texas Agricultural and Mechanical College, College Station.

Lactose content, conductivity, and freezing-point depression were determined on 480 fresh, raw milk samples from individual cows. Per cent lactose was determined by the A.O.A.C. method, using a Rudolph (Routine Model 60) double-field polarimeter. Conductivity readings at 37° C. were made with a conductivity bridge (Industrial Instrument Co., Model RC-16B) and accompanying conductivity cell, whereas the freezing point was determined with a Hortvet cryoscope. The correlaton beitween lactose content and freezing-point depression was 0.25; between conductivity and freezing-point depression, −0.33, and between lactose content and conductivity, −0.75. Considering freezing-point depression as the dependent variable, the multiple correlation involving the three variables was 0.33, the partial standard regressions of freezing-point depression on lactose content and conductivity being, respectively, −0.0034 and −0.3343. Thus, these data indicate that nothing is gained by measuring lactose content in addition to conductivity, if the objective is to estimate freezing-point depression, since lactose content is so highly correlated with conductivity.

M3. Influence of vacuum pasteurization upon the freezing points, total solids, and concentration of milk. J. T. LAZAR, JR., AND R. W. HENNINGSON, Clemson Agricultural College, Clemson, S. C.

Milk was pasteurized at 161, 165, 170, and 175° F. and treated at 22, 24, and 26 in. of vacuum in a DeLaval Vacu-Therm pasteurizer, to determine the amount of concentration of product that occurred. Milk was pasteurized also in a HTST pasteurized at these temperatures. The condensates from the vacuum-treated milk were measured and the concentrations of the samples were computed. Concentrations were determined also by comparing the total solids by the Mojonnier method and freezing points by the Hortvet method of the raw and vacuum-treated samples. Practically no concentration occurred in milk samples pasteurized at less than 175° F., unless the vacuum level was 24 in. or more. Concentration occurred at this treatment level and above. The ranges of concentrations were 1.28 to 1.96% for the condensate and 1.64 to 1.94% for the total solids. A rise occurred in the freezing points of samples treated at less than 175° F. and 24 in. of vacuum. Treatments above this level depressed the freezing points. As indicated by the total solids, there was some concentration in the HTST samples. To determine the amount of concentration due directly to vacuum and temperature treatment, the ap-

parent MTST concentration must be subtracted from the total concentration. The maximum concentration (as calculated on this basis) that occurred in the Vacu-Therm was 1.49%.

M4. Sedimentation of whole milk heated in the Mallory small-tube heat exchanger. H. K. Wilson and E. O. Herreid, University of Illinois, Urbana.

The rate of sedimentation of milk solids, principally proteins, is affected by the preheating temperature of the milk before sterilization. Portions of milk from the same batch were heated from 50 to 320, 300, 260, 220, 180, and 140° F. Average sedimentation rates after 66 days were 116, 52.3, 39.7, 44.1, 38.6, and 42.5, respectively, and raw milk was 23.7. Portions of another batch were heated from 140 to 320, 310, 300, and 290° F., the sedimentation rates were 84.9, 68.3, 44.1, and 33.1, and for raw milk, 25.4. All values must be multiplied by 10^{-11}. Nitrogen analyses indicated at sedimentation equilibrium that 20 to 30% of the proteins precipitated at pH 4.5 to 4.7 remained in the supernatant. The rate of protein separation at a force of $9{,}744 \times G$ was not affected by the temperature of storage, but was affected by the sterilization temperature. The serum protein nitrogen left in the supernatant in milk stored at 100° F. increased with decreased heating. In milk stored at 40° F., the serum protein nitrogen in the supernatant was uniformly lower. The Fisher-Stern ultracentrifuge was used in these studies.

M5. Effect of treatment of raw milk on heat-liberated hydrogen sulfide.* T. Kristoffersen, The Ohio State University, Columbus.

Observations have revealed that the amount of hydrogen sulfide liberated from milk by heat may vary widely, depending on the treatment. Determination of heat-liberated hydrogen sulfide was made by heating milk at 90° C. for 30 min. (Boyd and Gould, *Journal of Dairy Science,* 1957). The concentrations of hydrogen sulfide released from mixed-herd raw milk varied from 220 to 490 γ per liter. To determine the possible cause for these variations, fresh raw milk was incubated at 37° C. for 8 hr. Hydrogen sulfide determinations were made periodically. Results revealed a decrease in heat-liberated hydrogen sulfide during the first 45 min. of incubation, followed by an increase during the next 3 to 5 hr. and a considerable decrease during the remainder of the period. No appreciable bacterial growth occurred during the first 3 to 5 hr. of incubation. Accelerated growth occurred during the second decrease. Another factor affecting release of hydrogen sulfide from milk was low-temperature preheating. Milk was preheated at from 55 to 70° C. for 30 min., or at 63° C. for from 10 to 60 min. With either method, the more severe the preheating treatment the greater the decrease in heat-liberated hydrogen sulfide. The effect of preheating could be overcome by addition of Versene prior to preheating.

* Supported in part by funds from the American Dairy Association and the Ohio Dairy Products Research Fund.

M6. A study of factors which cause variations in the protein-reducing value of fluid milk. J. T. Cardwell and F. H. Herzer, Mississippi State College, State College.

Factors that cause variations in the protein-reducing substances (P.R.S.) of milk were studied with the modified procedure reported at the Southern Division of the American Dairy Science Association in 1957. The P.R.S. value of milk pasteurized by the vat method was greater than that pasteurized by the high-temperature, short-time method. The higher the heat treatment and/or the longer the holding period, the greater was the reducing value. Homogenized milk had a higher reducing value than unhomogenized milk, under the same conditions of heat treatment. The P.R.S. values increased with greater homogenization pressures. No relationship was evidenced between the fat content and the P.R.S. value of the milk. The P.R.S. values of milk from individual Jersey cows averaged greater than the values from either Guernseys or Holsteins. The addition of re-liquefied nonfat dry milk solids to fresh fluid milk increased the P.R.S. values in proportion to the amount of re-liquefied nonfat dry milk added, as determined by the modified procedure. The study was limited to 12% replacement.

M7. Isolation of milk phospholipids and determination of their polyunsaturated fatty acids. L. M. Smith and E. L. Jack, University of California, Davis.

Lipids were extracted from spray-dried buttermilks with equal volumes of hot ethanol, ether, and chloroform. After solvent removal, the lipids were re-extracted with a 50:50:1 ether-pentane-ethanol mixture, dried with sodium sulfate, and filtered. The bulk of the triglycerides was removed by filtration after precipitation of the phospholipids with acetone. Small amounts of phospholipids remaining in the glycerides were absorbed on a silicic acid column and, after elution with methanol, were added to the acetone insoluble material. The phospholipids were further purified by column chromatography. The amounts of polyunsaturated fatty acids were determined from ultraviolet absorption data obtained before and after isomerization. A representative sample of phospholipids (iodine value 31.6, 3.41% P, 3.85% N) contained, in percentages, 1.6 conjugated dienoic, 4.2 monoethenoid, 4.6 linoleic, 2.0 linolenic, 1.2 arachidonic, and 1.7 pentaenoic acids. The percentages of each acid in the total unsaturated fatty acids (with the corresponding percentages for a typical milk fat, given in parentheses) were: 11 (2.5), 27 (87.8), 30 (4.7), 13 (3.2), 8 (0.9), and 11 (0.9), respectively.

M8. Determination of ammonia and the lower volatile amine concentrations in milk by solvent extraction and gas-liquid chromatography.* C. L. HANKINSON AND D. D. COLE, Ohio Agricultural Experiment Station, Wooster, and The Ohio State University, Columbus.

Published methods for the determination of ammonia and lower volatile amines by gas-liquid chromatography employed celite as the immodile phase, undecanol and liquid paraffin as the liquid phase, and dry nitrogen as the eluting gas. Modifications consisting of (1) higher pressure and (2) progressively increasing temperature resulted in good resolution, with quantitative recoveries. Known concentrations of ammonia, N-methyl, N-ethyl, N-propyl, N-butyl, and N-hexyl amines were separated and titrated while the column temperature increased from 40 to 130° C. in 45 min. at a nitrogen pressure of 60 cm. of mercury. Ammonia and the lower volatile amines were extracted from 20 ml. acid milk filtrate, using a modified Roese-Gottlieb procedure. The filtrate was made alkaline and extracted three times, using a total of 150 ml. ethyl alcohol, 600 ml. ethyl ether, and 600 ml. petroleum ether. The combined extracts were acidified with HCl, evaporated to dryness as the hydrochlorides, and dissolved in ethyl ether. The free amines were released by alcoholic NaOH just before adding them to the chromatographic column. Analyses revealed variable concentrations of the lower volatile amines in milk.

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M9. A method for the determination of citric and lactic acids in dairy products. D. R. FRAZEUR, Purdue University, Lafayette, Indiana.

A three-section silicic acid chromatographic column was used with 2 ml. of water and 3 g. of silicic acid in the lower section; 8 ml. of 0.7 *N* H_2SO_4 and 12 g. of silicic acid in the middle section; and 4 ml. of 0.7 *N* H_2SO_4, 1 ml. of water, and 12 g. of silicic acid in the upper section. A 3-g. sample was used for the determination. The eluate was a water-saturated mixture of 40% butanol and 60% chloroform. The effluent was removed from the column in 75, 225, and 300-ml. fractions. A colorimetric procedure specific for hydroxy acids was used to determine the lactic acid which appeared in the first fraction. A titration procedure was used to determine the citric acid which appeared in the third fraction. A 97%, or better, recovery was obtained for concentrations of 0.020 to 3 γ of lactic or citric acid per gram of sample. Amino, fatty, and other organic acids present in the sample do not interfere with the determination. This method has been used successfully with ten dairy products. Very little preparation of sample is necessary.

M10. Distribution between the fat phase and the aqueous phase of certain antioxidants added to whole milk. J. MARTINEZ-MATEO, W. L. CHOATE, AND L. F. EDMONDSON, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

During studies of the effect of antioxidants on the keeping qualities of dried whole milk, it was found desirable to obtain information on the phase distribution of these antioxidants when added to whole milk. Recoveries of butylated hydroxyanisole (BHA) and nordihydroguaiaretic acid (NDGA) were made on washed and dried butteroil made from fresh whole milk containing from 0.003 to 0.05% antioxidant. It was found that from 70 to 90% of the BHA could be recovered in the butteroil, and less than 5% of the NDGA added appeared in the butteroil and butter serum fractions. Analysis of BHA in cream fractions obtained by siphoning off successive layers from gravity-separated milk indicated that the distribution of this antioxidant between phases was not dependent on the fat globule size. The effects of homogenization upon the distribution of these antioxidants will be discussed.

M11. Properties of interesterified butteroil. H. D. WEIHE AND G. R. GREENBANK, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

For use in cocoa butter and in some baked foods, a butter with modified physical properties is desirable. Research in chemical modification of other fats indicates that similar treatment of butter might result in the desired product. Butteroil was interesterified in an atmosphere of nitrogen under conditions leading to random interesterification at 55 and 85° C., using either sodium methylate or sodium potassium alloy as catalyst. Directed interesterification was carried out at from 25 to 30° C., using the latter catalyst. Changes in the physical properties of the butteroil as a result of random and directed interesterification are given, respectively, in the following data: capillary melting point from 34.0 to 43.0 and 44.0°; micropenetration (units of one-tenth mm.), at 10° C. from 27.6 to 19.7 and 18.0, at 15° C. from 43.0 to 22.6 and 24.2, and at 20° C. from 62.0 to 58.0 and 33.0; per cent solid phase by dilatometry at 10° C. from 45.6 to 54.4 and 32.2, at 25° C. from 12.2 to 22.0 and 30.5, and at 40° C. from 0.1 to 1.4 and 14.4. The interesterified butteroils were free of off-odors, but even after washing with warm water and heating for 1 hr. at 100° C., with nitrogen at 5 mm. pressure passing through it, the oil gave a slightly bitter flavor when homogenized with fresh skimmilk. The causes of, and means for eliminating, this flavor are being studied.

M12. Characterization of the volatile and nonvolatile fatty acids obtained by a Reichert-Meissl-Polenske distillation. ARUN SENGUPTA,

W. F. SHIPE, AND A. C. DAHLBERG, Cornell University, Ithaca, N. Y.

The fatty acids from butterfat and admixtures of butterfat with other fats were separated by the conventional Reichert-Meissl-Polenske distillation. The volatile fatty acids were separated by chromatographic analysis. The Reichert-Meissl fraction contained butyric, caproic, and caprylic acids. Caprylic and capric acids were the principal components of the Polenske fraction, but it also contained some higher and lower acids. The nonvolatile fatty acids were recovered from the distillation residue by transferring it to a 50% cream-test bottle and centrifuging in a heated Babcock centrifuge. An aliquot of the fatty acids was fractionated with urea. The refractive indices of the original fatty acids, and the fatty acids in the filtrate and precipitate from the urea treatment, were determined. The refractive indices of these three fractions provided a basis for identifying the type of fat. A comparison of the Reichert-Meissl and Polenske values with the refractive indices of the nonvolatile fraction may serve to detect lower levels of adulteration than can be detected by any single value.

M13. Vitamin B_{12} in whey and casein fractions of milk separated by coagulation with acid and rennet. E. B. COLLINS AND MAKOTO YAGUCHI, University of California, Davis.

Vitamin B_{12} was determined by the U.S.P. microbiological assay method, using *Lactobacillus leichmanii* ATCC 7830 and electrometric titration to pH 7.0. Before samples to be tested vere diluted to the desired volume, they were adjusted to pH 6.0. Wheys were prepared from pasteurized skimmilk that contained 0.43 γ of vitamin B_{12} per 100 ml. Coagulation with glacial acetic acid produced whey containing 0.19 γ of vitamin B_{12} per 100 ml.; coagulation with rennet produced whey containing 0.28 γ per 100 ml. These amounts were 44 and 65%, respectively, of the amount of vitamin B_{12} in the original skimmilk. The total solids contents of the wheys were 6.78 and 6.86%, respectively. Examination of two additional acid wheys, three cottage cheese wheys, two additional rennet wheys, and two Cheddar cheese wheys indicated distributions of vitamin B_{12} similar to those above. Adjustment of rennet whey to pH 4.6 did not change its vitamin B_{12} content. The larger amount of vitamin B_{12} in rennet whey suggests that rennet may cause slight hydrolysis and/or separation of bound vitamin B_{12} from casein.

M14. Observations on bulk milk quality on truck vs. individual farm tank samples. H. V. ATHERTON, University of Vermont, Burlington.

The quality of farm bulk-cooled milk from individual bulk tanks has been compared with that of samples taken of the combined deliveries in the bulk-pickup truck, using several common laboratory procedures. Studies were made on 13 truckloads of milk, representing 88 tank collections on seven different routes. The weighted average of values determined for individual farm samples was compared with the results of samples taken from the truck at the milk plant. Results showed that the milk flavor (primarily feed) and protein stability were not changed by conditions of pickup. Standard plate, heat resistant, and psychrophilic counts were generally higher on the truck sample than the weighted average of individual deliveries would indicate, but these differences were never bacteriologically significant. This study indicated milk temperatures will increase by from 1 to 2° F. during transport from farm to plant. It would appear that creaming ability, already lowered by conditions of bulk cooling, was further depressed by pumping, and transport in the truck.

M15. Quality of manufacturing-grade milk handled in farm bulk tanks. W. S. LAGRANGE AND F. E. NELSON, Iowa Agricultural Experiment Station, Ames.

Manufacturing-grade milk from 106 producers was examined for standard plate count at 32° C., count of psychrophilic bacteria, thermoduric count, reazurin reduction class, and organoleptic score. Of the 232 samples, 74 had plate counts exceeding 2,000,000/ml and 56 others had counts in excess of 500,000/ml. The count of psychrophilic bacteria frequently was nearly as high as the standard plate count and sometimes exceeded it considerably. Occasionally, the thermoduric count exceeded 10,000/ml. The resazurin reduction test failed to detect many samples with high plate counts. In only a few instances were organoleptic defects attributable to high counts of bacter. Of 62 farms visited, only ten had both a stationary wash-vat and a water heater. Plate counts on only three of the 14 samples from these ten producers exceeded 500,000/ml and only one count exceeded 2,000,000. Among the producers without these two facilities, only a few were able to consistently produce milk with plate counts below 500,000/ml. Other shortcomings in facilities and management were common. Bulk tanks alone are not the answer to quality of manufacturing-grade milk.

M16. Observations on bacteria counts of farm bulk-cooled milk. H. V. ATHERTON, University of Vermont, Burlington.

Recently discussions of milk quality have emphasized the role of psychrophilic and facultative-psychrophilic bacteria in the quality control of milk handled under the bulk-cooling system. Several incubation procedures are used in the dairy industry to determine the bacterial count of farm bulk-cooled milk. The current edition of Standard Methods permits two different media for the Standard Plate Count. There is a need to ascertain whether the several procedures for obtaining bacterial counts on

bulk milk give equivalent results throughout the period of raw storage. Twenty-four samples of bulk-cooled milk were divided into eight replicates for daily study during six days of storage at 40° F. Analyses were made following incubation at 32° C. for 48 hr., 20° C. for 48 hr., 20° C. for 72 hr., 5° C. for seven days, and 5° C. for ten days, using each of the media approved in Standard Methods (tenth edition). Results of this study indicate that counts made on milk samples of a given age are quite comparable, regardless of the plating medium or temperature of incubation. There is no advantage to incubating plates at 5° C. for ten, rather than seven days, or at 20° C. for three, rather than for two, days.

M17. Preserving the activity of lactic cultures. BURDET HEINEMANN, Producers Creamery Co., Springfield, Mo.

To both cheese and butter cultures, 0, 10, and 20% sterile glycerine was added after ripening. The cultures were then held at 35, 5, and −20° F. for periods up to 6 mo. Upon removal from storage, the cultures were used to inoculate autoclaved milk at a level of 1%, and titratable acidity was measured after incubation for 16 hr. Cultures containing 20% glycerine remained active up to 2 mo. of storage at 35° F. and up to 6 mo. of storage at 5 and −20° F. The activity of cultures containing no glycerine was appreciably decreased during the same storage conditions.

M18. Production, distribution, and use of frozen, active lactic acid cultures. J. C. SIMMONS AND D. M. GRAHAM, Clemson Agricultural College, Clemson, S. C.

A procedure which minimizes contamination, eliminates daily transfers, and offers an active culture that will possess uniform characteristics has been studied. The procedure for using these cultures consists of inoculating a suitable medium with an active culture, freezing the culture immediately in a −20° F. room, thawing the culture in a 70° F. water bath, incubating for 16 hr. at 70° F., then using this coagulated mother culture to prepare bulk starter. Studies of these cultures indicate that the organisms produce normal levels of acid and flavor in a medium of nonfat dry milk solids reconstituted at the rate of 10%. If the medium is enriched by the addition of extra milk solids or 10% V8 Juice, an increase in acid production is observed. Four commercial starters were frozen, stored at −20° F. for one month, reactivated, and tested for activity by the Leber resazurin and Horrall-Elliker acid production tests. All strains responded equally well when reactivated following storage at −20° F. No difference in activity was observed between the reactivated frozen cultures and the cultures transferred daily. Several of these frozen cultures, used in preparing bulk starter for cottage cheese, developed acidity in the cheese comparable to bulk starter prepared with fresh cultures. The procedure and finished cheese were normal by all criteria.

M19. Effect of temperature on acid production by Streptococcus lactis and Streptococcus cremoris. J. WOLK AND R. P. TITTSLER, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The amounts of acid produced by nine strains of *Streptococcus lactis* and 14 strains of *Streptococcus cremoris* at different temperatures and combinations of temperatures from 82 to 105° F. were determined. Activity tests were made in sterilized skimmilk for 6 hr. The temperature was held constant throughout, or held constant for a definite period and then raised quickly to one of the higher temperatures. Acid production was measured every 2 hr. either by titration or by pH determinations. Production of acid by both species was greatest at 94° and much less at 100 and 105° F. Some variation was noted among strains of both species. The results emphasize a need for properly adjusting starter-temperature relationships in cheese-making and provide useful data for establishing such relationships. They also show that the use of two temperatures may be more desirable than one in conducting activity tests.

M20. Acceleration of cottage cheese making by using starter culture stimulants. M. L. SPECK AND R. A. LEDFORD, North Carolina State College, Raleigh.

Earlier reports have shown that an aqueous extract of pancreas tissue contained peptides that stimulated the development of various lactic acid bacteria in milk. The present study concerns an extension of the work to commercial types of milk fermentations. Cottage cheese was made from nonfat dry milk solids reconstituted to 10.5% solids in 2-liter batches, using 5% inoculum, 0.02% $CaCl_2$, rennet (1 ml/1,000 lb. milk), and an incubation temperature of 90° F. The time required for the milk to coagulate and reach proper acidity for cutting was reduced approximately 17–41% with concentrations of 0.015–1.0% of pancreas extract solids added to the milk. Concurrent with the decrease in ripening time was a decrease in the cooking time of 12–92%. Similar results were obtained using 30-gal. batches of milk. The cottage cheese made with stimulants had no foreign flavors and had a shelf-life comparable to cheese made with unfortified milk.

M21. The influence of stimulatory peptides on proteolysis of milk by lactic streptococci. M. L. SPECK AND W. T. WILLIAMSON, North Carolina State College, Raleigh.

Numerous studies have shown that the growth of many lactic streptococci in milk are stimulated by various peptide-rich extracts. The role of peptides in stimulating growth has re-

mained unexplained. In the present study, mixed-strain starter cultures showed less proteolysis in milk when the milk was fortified with stimulatory peptides, such as contained in pancreas extract. There was an inverse relation between the amount of proteolysis and the concentration of the pancreas extract in the milk. In addition, the curd tension measurements of cultures at 0.85% titratable acid were greater in milk fortified with pancreas extract than in unfortified milk. The data indicated that the presence of stimulatory pepttides in milk precluded the necessity for cultures to hydrolyze milk proteins before initiating growth. The relation of this study to certain cheese-making steps will be discussed.

M22. Inhibitory effect of nisin upon various organisms. K. M. SHAHANI, University of Nebraska, Lincoln.

Two nisin-producing strains of *Streptococcus lactis* varied in their ability to produce antibiotic activity. In a milk medium containing the nisin-producing organisms, acid production by two strains of *Streptococcus thermophilus* was delayed, but their total acid production was not affected appreciably. Nisin did not inhibit a mixed *Streptococcus* and *Lactobacillus* culture, and two strains each of *S. lactis* and *S. thermophilus*. Both cultures of the antibiotic-producing organisms inhibited only two of 11 strains of *Staphylococcus aureus*. The antibiotic was found to be active only against the coagulase-negative and weak coagulase-producing *Staphylococci*. Nisin was less inhibitory to dairy cultures than penicillin, streptomycin, aureomycin, or erythromycin.

M23. A test for detecting lactic bacteriophage using 2,3,5-triphenyltetrazolium chloride. B. J. LISKA, University of Florida, Gainesville, AND H. E. CALBERT, University of Wisconsin, Madison.

2,3,5-Triphenyltetrazolium chloride (TTC) was used to develop a test for detecting bacteriophage inhibition of lactic cultures. The principle of the test is based upon the inhibition of microbial reduction of TTC by the bacteriophage. Bacteriophage action is indicated when inhibition of normal TTC reduction by the lactic cultures occurs. Controls, known to be free of bacteriophage, are established to determine the degree of inhibition. The results obtained using the TTC test were compared to results using an accepted litmus milk test requiring 12–16 hr. to perform. The TTC test in 4.5 hr. time yielded results comparable and in some cases better than the litmus milk test. Phage was detected in filtrate dilutions up to 10^{-10} or having a titer of ten with the TTC test, when using single-strain *Streptococcus lactis* cultures and specific phage strains. When using multiple-strain lactic cultures, some cases of phage inhibition detected after 4.5 hr. of incubation with the TTC test were not evident after 12–16 hr., using the litmus milk test. Several factors which affect the sensitivity of the TTC test are: *(1)* amount of inoculum; *(2)* concentration of TTC; *(3)* time of TTC addition, and *(4)* length of time for the test.

M24. Association between Bacterium linens and an oxidative type yeast in the ripening process of surface-ripened cheeses. R. L. MAGINNIS AND J. F. CONE, The Pennsylvania State University, University Park.

A study was made of an endocellular polypeptidase extracted from an oxidative type yeast (*Trichosporon* species) isolated from Trappist-type cheese. This enzyme was capable of further breaking down at TCA soluble polypeptide formed by the action of *Bacterium linens* on a 2.5% casein medium prepared by the methods of Peterson (1948). A crude autolysate containing the yeast polypeptidase was partially purified by precipitation of the active fraction with two volumes of ethanol to one of autolysate. The activity of the enzyme on the polypeptide preparation was measured by titration with alcoholic HCl of the enzyme-treated substrate in acetone, according to a modification of the Linderstrom-Lang method. Preliminary studies on pH optimum showed little activity below 6.0. There was a broad peak at pH 6.6 to 7.0. The optimum temperature was 30° C. A study of the effect of activators and inhibitors showed that Zn and Mn ions gave appreciable activation.

M25. Effect of penicillin upon the morphology and gram-staining characteristics of Streptococcus lactis and Streptococcus thermophilus. R. W. BAUGHMAN AND F. E. NELSON, Iowa State College, Ames.

Morphological changes induced by various concentrations of penicillin were studied at temperatures of 21, 32, 37, and 45° C. Morphological changes were more rapid at higher penicillin concentrations. Cells became greatly elongated and rather irregular in shape in the presence of the antibiotic. The cells of *S. thermophilus* tended to form extremely long chains, even in concentrations of penicillin as low as 0.03 unit per milliliter. The typical gram-positive staining reaction of *S. lactis* and *S. thermophilus* was reversed upon exposure of the cells to various concentrations of penicillin. As the concentration of the penicillin was increased, this phenomenon was more pronounced. However, even at levels as low as 0.05 unit per milliliter this reversal was apparent at 45° C. within one hour when using *S. thermophilus*.

M25A. Taxonomy of carbon dioxide–producing lactic streptococci found in mixed-strain starter cultures. W. E. SANDINE, P. R. ELLIKER, AND A. W. ANDERSON, Oregon Agricultural Experiment Station, Corvallis.

The causes of the floating curd defect in cottage cheese have been studied. It has been

demonstrated that this defect often is caused by high carbon dioxide–producing lactic acid streptococci found in mixed-strain starter cultures. When these types became the dominant organisms in a mixed culture, a high gas-producing starter culture resulted. Single strains isolated from these high gas-producing, mixed-strain starter cultures, on the basis of their rate of acid and carbon dioxide production, were placed in four general groups, namely: high acid–high gas, low acid–high gas, high acid–low gas, and low acid–low gas. A taxonomic study was made properly to classify each one. Known species of lactic acid streptococci and associative *Leuconostoc* organisms that may be used in controlled dairy fermentations were included for comparison with organisms isolated from mixed-strain cultures. Each organism was examined for at least 34 different cultural reactions. The high acid–high gas and the low acid–high gas types were identified as *Streptococcus diacetilactis,* the low acid–high gas types as *Streptococcus cremoris,* and the low acid–low gas types as *Leuconostoc* species or slow acid-producing strains of *S. cremoris. Streptococcus lactis* organisms were not found.

M26. Some observations on lipase activity. B. L. Herrington and E. S. Guthrie, Cornell University, Ithaca, New York.

Many of the factors responsible for induced rancidity in cow's milk have been identified, but less is known about factors responsible for spontaneous rancidity. The stage of lactation does not seem to be a factor affecting induced rancidity, but it may be a factor in spontaneous rancidity. Acid degree values have been determined at regular intervals on the milk of 18 individual cows at zero-time and after 24 hr. of refrigeration. The experiment will continue for entire lactations. It is already apparent that lipase activity is very low in milk secreted during the first week of lactation.

M27. Effect of light, agitation, acids, and rennet upon lipolytic activity in milk. B. J. Demott, University of Tennessee, Knoxville.

Exposure to sunlight for one hour of homogenized milk in clear or amber-colored glass bottles resulted in slightly lower free fat acidities than milk not so exposed. The amber-colored bottle protected the enzyme(s) only very slightly when its activity was determined immediately, but protection was apparent after the milk has been stored 24 hr. at 40° F. The protective effect of the amber bottle against the development of oxidized flavor was demonstrated. Mixtures of pasteurized whole milk and raw skimmilk, one of which was agitated in a Waring Blendor, shows that agitation of the substrate is more effective in producing lipolysis than a like agitation of the enzyme(s). Precipitation of casein by use of HCl, lactic acid, or rennet showed the latter to have the least destructive effect on the lipase(s).

M28. Acid values and 1-monoglyceride content of normal and rancid milk. R. G. Jensen and M. E. Morgan, Storrs Agricultural Experiment Station, Storrs, Connecticut.

This study showed that the 1-monoglyceride content of fat from milk increased as the milk became rancid. Five samples of milk were prepared by mixing raw and homogenized milk in equal portions. The samples were analyzed for acid degree values (ADV) (silica gel method) and 1-monoglycerides, immediately after preparation and after 24 hr. of refrigerated storage. The fat for the monoglyceride analysis was obtained by using a silica-gel extraction method similar to the ADV procedure. The monoglyceride contents were estimated by colorimetric determination of the formaldehyde formed from the oxidation of 1-monoglycerides by periodic acid. The average ADV of the normal samples was 1.67 (1.43–1.90) and the average 1-monoglyceride content (reported as m*M* per 100 g. fat) was 0.304 (0.208–0.418). The average ADV for the 24-hr. samples was 11.56 (10.16–12.31) and the average 1-monoglyceride content was 0.669 (0.510–0.895). Theoretically, there could be 5.78 m*M* of 1-monoglycerides for an average ADV of 11.56. The level of 1-monoglycerides found may be low, because of the presence of diglycerides and 2-monoglycerides (through which fat hydrolysis proceeds with pancreatic lipase). 2-Monoglycerides are not oxidized by periodic acid. The 1-monoglyceride found probably arise from isomerization of 2-monoglycerides.

M29. Distribution and partial purification of milk lipases.* Z. Saito, W. J. Harper, and I. A. Gould, The Ohio State University, Columbus.

Using activities at pH 7.0 and pH 8.6 as indicative of two different lipases, the distribution of these lipases in cream, skimmilk, and sediment obtained by centrifugation at 6,000 × gram for 30 min. has been studied. Lipase activity at both pH levels was observed in all three fractions. However, the relative proportion of activity in the different fractions varied. Slight purification of milk lipases was achieved by centrifugation. The extent of purification varied markedly, according to season of the year and to the individual milk supply. Generally, increases in specific activity of about three- to fivefold of both pH 7.0 and 8.6 lipases were obtained. Butanol extraction, phosphate-gel and ethanol fractionation were utilized for further purification of the milk lipases. Acetone and magnesium sulphate fractionations were not successful in further purification of the milk lipases. Paper chromatographic analysis of the partially purified lipase preparation, using an acetone-water solvent system, revealed separation of lipase ac-

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tivity at pH 7 and at pH 8.6. The lipase had Rf values of 0.75 and 0.9, respectively, and were separated from the majority of the other proteins in the preparation.

M30. Rates of lipolysis in milk as affected by duration of activation by shaking. N. P. TARASSUK AND M. YAGUCHI, University of California, Davis.

Freshly drawn milk from individual cows was activated by shaking at 37° C. for periods up to 6 hr. The rates of lipolysis during shaking, and after shaking and aging in cold, were investigated. The velocity of lipolysis is highest during the first hour of activation by shaking, it decreases thereafter, and after 2 hr. of shaking it remains fairly constant at about one-third of its initial (first-hour) rate. This initial rate varies with different milks and accounts for existing variation in susceptibility to lipolysis. The extent of lipolysis in activated milks developed in storage (total F.F.A. titration—F.F.A. titration right after shaking) increases rapidly with the increase in time of activation by shaking, and reaches a maximum value in milk shaken an average of 1 hr. The value thereafter remains nearly constant on shaking for as long as 6 hr. and it is of about the same magnitude for all milks tested. These data, on velocity of lipolysis during shaking, indicate that absorption of lipase on fat globules is complete on activation by shaking for 1 hr.

M31. Possible role of xanthine oxidase in spontaneously oxidized milk. A. E. WOODS, L. W. AURAND, AND W. M. ROBERTS, North Carolina State College, Raleigh.

Studies were made to determine the role of oxidizing enzymes, normally present in milk, in the development of spontaneously oxidized milk. Xanthine oxidase, peroxidase, catalase, and their substrates, in varying concentrations, were studied. The milks used for this study were raw, batch-pasteurized, and heat-inactivated (76.6° C. for 30 min.). Results of these studies show that peroxide and catalase, together with varying levels of substrate, had no effect on the development of oxidized flavor. Conversely, xanthine oxidase when added at optimum levels of activity, gave rise to an oxidized flavor. Hypoxanthine in varying concentrations prevented the development of the off-flavor. Thus, an enzyme mechanism is suggested; namely, xanthine oxidase acting upon an unknown substrate(s) gives rise to the oxidized flavor principle.

M32. Study of the phosphatase test for milk pasteurization. J. B. MICKLE, P. J. MAGNINO, JR., AND R. D. MORRISON, Oklahoma State University, Stillwater.

Milk was pasteurized at 62.5 and 76.7° C. and tested for phosphatase activity immediately after pasteurization and again after 24 hr. of incubation at 32.2° C. In Trial I, the phosphatase test was run in the customary manner. Trial II was a repetition of Trial I, except that a bioassay technique was used in analyzing the samples. The minimum time of pasteurization was determined at each heating temperature and this was found to vary. This was particularly true at 76.7° C., where the minimum pasteurization time was 1 min., 56 sec. in August, 1957, and increased to 2 min., 6 sec. during December, 1957.

Phenol values of 3.6 ± 1.3 p.p.m. were established as the minimum for milk pasteurization in Trial I. In this trial, three of 16 samples appeared to be pasteurized after heating, but according to the phosphatase test were underpasteurized after incubation. However, no general trend toward higher or lower phosphatase values after incubation was established. In Trial II, ten of 13 samples gave higher values after incubation. However, these increases were usually smaller than the experimental error of the first trial.

M33. Stability of disodium phenyl phosphate in carbonate-bicarbonate buffer. R. W. HENNINGSON, Clemson College, Clemson, S. C.

A major practical impediment to the routine use of any phosphatase method is the instability of the disodium phenyl phosphate. Samples of this substrate were prepared in carbonate-bicarbonate (Cornell) buffer and treated and stored in the following manner: Sample No. 1 was unheated, $CHCl_3$-preserved, and stored at 20° C., No. 2 was unheated and stored at 5° C., No. 3 was unheated and stored at 20° C., No. 4 was heated to 85° C. for 3 min. and stored at 5° C., No. 5 was heated to 85° C. for 3 min. and stored at 20° C., No. 6 was autoclaved at 120° C. for 10 min. and stored at 5° C., and No. 7 was autoclaved for 10 min. at 120° C. and stored at 20° C. Samples were removed from storage at regular intervals and tested by adding N,2,6-Trichloro-*p*-quinoneimine (CQC) to the sample. Samples 1 and 3 showed 10 γ phenol after 1 wk. Sample No. 2 showed 5 γ after 2 wk. None of the other samples showed any phenol after 1 yr. of storage. At this time, a fresh buffer substrate was prepared and tested with stable, stored samples, using 0.04% raw milk, for sensitivity. All showed 8 γ phenol. Samples 1, 2, and 3 showed greater than 40 γ phenol after 1, 4, and 2 mo. of storage, respectively. Heated buffer-substrates used routinely over a period of 1 yr. showed no instability, when care was used in pipetting. Results indicate that heating the buffer-substrate maintained sensitivity, and kept it in a stable form for routine use, eliminating the necessity for preparing fresh solutions each time tests were to be made.

M34. A method to improve the sinkability of dried whole milk. D. H. BULLOCK AND W. C. WINDER, University of Wisconsin, Madison.

A simple test has been devised to estimate the

per cent of a milk powder sample which sinks after being placed on a quiescent water surface for a given period of time, usually 30 sec. Results are expressed as per cent sinkability. Studies to improve this sinkability property have been conducted on conventional whole milk powder and, in particular, on high-vacuum dried whole milk. Whole milk powder, in general, exhibits greatest sinkability when first removed from the drier. Much of this property is lost during the first day or two of storage. By subjecting the powder to a specific post-drying temperature treatment, the initial sinkability of the fresh powder could not be retained but could be improved considerably. The following whole milk powders exhibited marked increases in sinkability after the temperature treatment. The tests were made 48 hr. after the treatment. High vacuum–dried powder increased from about 20% to from 70 to 90%; spray dried powder, from 10 to 40%; aggregated spray-dried powder, from 17 to 70%, and roller-dried powder, from 7 to 17%. Subsequent tests on high vacuum–dried powder showed that the property was not lost, even after months of storage.

M35. Effect of raw milk quality on the flavor stability of dried milk. U. S. Ashworth and C. C. Prouty, State College of Washington, Pullman.

Experimental lots of spray-dried whole milk powder were prepared from raw milk having poor quality due to the presence of the following types of bacteria: *(1)* acid formers, *(2)* proteolytics, *(3)* lipolytics, and *(4)* psychrophiles. After preheat treatments of 175° F. for 10 min., the samples were spray-dried along with controls from the same bulk milk. The powders were stored at 45 and 100° F., both air- and nitrogen-packed for a total of 8 mo. Sample cans were taken from storage each 2 mo. for flavor evaluation and for chemical and bacteriological testing. The results from 170 lots of powder show that stale flavor develops in the powders, independently of the quality of the raw milk, and overshadows most flavor defects resulting from drying poor-quality milk within certain limits. In the freshly prepared powders after reconstitution, the flavor panel could significantly detect a drop in pH of 0.2 unit caused by bacteria. It could also detect proteolysis sufficient to give free tyrosine values over 1 mg. per g. of milk protein and lipolysis, sufficient to produce a value of two for the free fatty acid titer. The temperature of storage of the powders had a greater influence on flavor stabiltiy than did the bacterial quality, as measured by the standard plate count or by differential counts for acid formers, proteolytics, lipolytics, and psychrophiles.

M36. Identification of some compounds affecting the flavor of nonfat dry milk. Richard Bassette and Mark Keeney, University of Maryland, College Park.

Nonfat dry milks (NFDM), particularly some of the instant dry milks, are sometimes criticized for having cereal, hay, malty, and stale types of flavor. Study of the volatiles recovered by low-temperature vacuum distillation of reconstituted instant NFDM demonstrated that carbonyl compounds and methyl sulfide played a prominent role in these off-flavors. The following carbonyl compounds have been identified via their 2,4-dinitrophenylhydrazones: diacetyl, furfural, acetaldehyde, acetone, 2-methylpropanal, 3-methylbutanal, hexanal, and nonanal. Several other volatile carbonyls are present in cereal-stale flavored NFDM and evidence is being accumulated for their identification. The compounds thus far identified substantiate previous suggestions, that Maillard-type browning is involved in cereal flavor development. The mechanism of flavor development will be discussed in light of the identified compounds.

M37. Further investigations on the stale-flavor components in dried whole milk. W. W. Nawar, H. E. T. Dall, A. S. Ganguly, S. H. Lombard, and R. McL. Whitney, University of Illinois, Urbana.

The CCl_4-vapor distillate of stale butteroil extracted from stale dried whole milk contains stale-flavor components, and its infrared spectrum indicates the presence of carbonyls. Therefore, an extraction of stale butterfat with Girard's-T-reagent was successfully attempted. Attempts to further fractionate the Girard's-T-extract by alkaline or sodium-bisulfite extraction resulted in loss of the stale flavor. To determine whether alkalinity, as such, would prevent the development of stale flavor, dried whole milks prepared from condensed milks adjusted to different pH levels were stored under varied conditions. Alkalinity was found to delay the appearance of stale flavor; however, even at pH 7.5, the alkaline flavor frequently was detected by the judges. Further work on the fractionation of the stale-flavor components revealed that there are at least two different stale-flavor components: one, Girard's-T-extractible but nonvolatile, and the other, volatile but non-Girard's-T-extractible. The relative distribution of the stale flavor between thees two components seems to vary with the character and previous history of the dried whole milk under investigation. The Girard's-T-extractible-nonvolatile fraction has been further fractionated by paper chromatography into a stationary and a mobile component, both of which contain stale-flavor components.

M38. Migration of phosphatides in processing foam- and spray-dried whole milk. G. R. Greenbank, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Many investigators who have studied the oxidative deterioration of dry whole milk have proposed that the phosphatides trigger the oxidation of the glycerides. In further experiments on the relationship of milk phosphatides to the stability of dry whole milk, data are reported that show the phosphatides (as measured by those phosphorus-containing materials which are soluble in fat solvents) migrate from the fat phase (cream) into the water phase (nonfat milk) as processing proceeds through pasteurization, condensation, homogenization, and drying. Any form of agitation causes the phosphatides to move from the fat globules into the skimmilk. In some batches of foam-dried whole milk the nonfat phase of the reconstituted milk contained twice as high a concentration of phosphatides as was found in the control milk. The data are discussed with regard to the light they throw on processing methods which have been found by these laboratories and industry to retard deterioration. A new and simple method of comparing the phosphatide content of different samples of butterfat is described.

M39. Effect of superheating on the baking properties of nonfat dry milk. L. V. Rogers, H. A. Anderson, and C. F. Hufnagel, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

In preparing nonfat dry milk (NFDM) especially for use in baking, skimmilk normally receives a high-temperature forewarming treatment, as this gives a product with good baking properties. However, since NFDM has varied uses and a wide range of conditions prevail in its manufacture, a study was made of the effect of these conditions, especially superheating, on the baking properties of spray-dried NFDM. To cover the range of commercial practices, three forewarming temperatures, three solids concentrations in the condensed skimmilk, and three superheating conditions were used. The NFDM was baked into white pan-bread at the 6% level, based on the flour, and the effects of the processing steps on such factors as water absorption of the dough and loaf volume were determined. In general, undenatured whey protein nitrogen in the NFDM decreased with the extent of the forewarming treatment of the skimmilk and the severity of superheating of the concentrate following low forewarming of the skimmilk. The baking properties of NFDM receiving the optimum forewarming treatment were not further improved by superheating of the skimmilk concentrate. Superheating the skimmilk concentrate for 30 min. at 175° F. imparted satisfactory baking properties to the NFDM, where an inadequate forewarming treatment was applied.

M40. Free fat in foam-dried whole milk. A. Tamsma, L. F. Edmondson, and H. E. Vettel, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Concentrated milks of 50% T. S. were foamed and dried in a vacuum shelf drier, and comminuted through a 20-mesh screen. Methods were developed for determination of free fat by extraction with carbon tetrachloride and of that fat readily separated by centrifugation of the reconstituted product. Large increases in free fat of from 10 to over 90% of the total fat resulted from aging and lactose crystallization of the concentrate. This effect was reversed by heating immediately before drying. Free fat decreased slightly with increased homogenization pressure. Free fat contents less than 10% of the total fat were obtained by control of these factors. Variations in free fat up to about 40% did not affect dispersibility of the dry milk or the state of dispersion of the fat in reconstituted milk. At high free-fat levels (40–95%), the dispersibility decreased and part of the fat was readily separated by centrifugation. Free fat increased with decrease in particle size. A fraction obtained by comminution through a 40-mesh screen, followed by removal of the finest particles by sieving on a 60-mesh screen, was similar in free fat, more dispersible, and much higher in bulk density, when compared to the product obtained by comminution through a 20-mesh screen.

M41. High milk-protein bread. L. V. Rogers and Helen H. Welton, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Satisfactory bread containing 25% nonfat dry milk based on the flour weight, or 20% by content, has been experimentally produced without major changes in customary bread production. The bread is typical of white pan-bread, except for a firmer crumb and a heavier and darker crust. Loaf volume is about normal. Taste evaluations showed this bread to have an outstanding milk flavor and excellent palatability. Nutritional studies are incomplete, but excellent protein quality is expected.

M42. A study of the processes of gelation, flocculation, and caramelization as related to heat stability in concentrated milk. R. N. Giroux, Ecole de Laiterie, Provinciale, St. Hyacinthe, Que., and H. E. Calbert and A. M. Swanson, University of Wisconsin, Madison.

Ultra-high temperature sterilization gives products of better flavor and color; however, gelation and fat separation are limiting factors. Using the proposed method, these factors were found to be associated with the heat stability phenomenon. The accelerated gelation-flocculation-caramelization test (G.F.C.) is based on the observation, by a procedure described in Paper M49, 52nd Annual A.D.S.A. meeting, Stillwater, Oklahoma, 1957, of viscosity changes upon quiescent heating of concentrated milk.

The results are presented in the form of a graph, relating viscosity to time of heating. The typical G.F.C. curve represents three processes: gelation, flocculation, and caramelization. Gelation is characterized by a reversible gel development up to a high maximum viscosity. Flocculation is characterized by an irreversible destruction of the gel to a minimum basic viscosity and is accompanied, among other things, by the loss of the solid suspension stability. Caramelization, as observed visually, is particularly characterized by an increase in viscosity which progresses at an accelerated rate. The addition of phosphate and calcium salts had a striking effect upon the G.F.C. curve. Although the general pattern remained the same, the intensity of the response was greatly modified.

M43. Ultra-high temperature sterilization and aseptic canning of concentrated milk with pilot equipment. G. H. HARTMAN, White House Milk Co., Inc., Manitowoc, Wisconsin, AND D. V. ALSTRAND, American Can Co., Maywood, Illinois.

Concentrated milk (2-1) was sterilized by a pilot tubular heat exchanger, homogenized at 3,500 p.s.i. after partial cooling, followed by additional cooling and aseptic canning. Samples were evaluated for flavor by multiple comparison procedure, using dilution technique by from ten to 12 judges and by direct scoring, using the proposed A.D.S.A. dry milk scorecard. Fresh canned milk diluted with 50% market milk rated slightly different in flavor from market milk. Preheating variables showed no significant difference in flavor. Reconstituted milk was slightly more viscous, equal in color, and scored several points lower in flavor than market milk. Heated flavors declined after from 8 to 12 wk. of storage at 40° F. and storage flavor developed thereafter. None gelled after 20 wk. Fat separation and precipitates were not detected after 20 wk. for most samples. Samples stored at 80° F. showed a slight storage flavor at 4 wk. Some lots held at 100° F. gelled in 8 wk., but others did not after 20 wk. Viscosity dropped slightly during the first month of storage, then remained constant. This test was useless in predicting gel formation. Milk not homogenized after sterilization was chalky, tactual, and viscous. Flavor stability is the most important factor, if this type of milk is to be marketed for beverage use.

M44. Factors pertaining to the stability of ascorbic acid and vitamin B_6 in modified milk products. O. F. GARRETT, R. W. SHARP, AND JEAN KLECKNER, M & R Dietetic Laboratories, Inc., Columbus, Ohio.

The stability of ascorbic acid and vitamin B_6 in modified infant-feeding milk products has been studied. Processing losses of B_6 in the powder were about 23%. Processing losses of the natural forms of B_6 in the sterilized liquid prior to sterilization were about 14%; sterilization losses were 25% of the natural forms and 10% of the synthetic form of B_6; sterilization losses of ascorbic acid were about 30 to 35%. Storage losses of ascorbic acid in the sterilized liquid were measurable, but B_6 was stable. Internal can surfaces of plain tin, "corn" enamel, and epoxy resin enamel did not influence the stability of ascorbic acid during sterilization and storage of the concentrated liquid, but losses of B_6 were 5 to 10% higher in the enameled cans. In terminally autoclaving the infant-feeding formula, destruction of B_6 was arithmetically proportional to the length of heating and semilogarithmically proportional to the temperature of heating. Losses at 250° F. for 5 min. and at 230° F. for 10 min. were comparable.

M45. Relation of type of fat in modified sterilized milk products to stability of vitamin B_6 and to physiological response when fed to rats. L. S. BAUR AND O. F. GARRETT, M & R Dietetic Laboratories, Inc., Columbus, Ohio.

Differences in the stability of vitamin B_6 in modified sterilized milk products containing six different single fats and one fat mixture were not significant. Three rat-feeding experiments were conducted in which the modified milk products were used. In the first experiment, the modified products contained nutrients that were physiologically balanced for infant feeding in which over 50% of the solids consisted of lactose. The fats in the four products studied were, respectively, milk fat alone, corn oil alone, cocoanut oil alone, and a fat mixture having a 50:50 ratio of saturated and unsaturated fatty acids. In the second experiment, the modified products contained nutrients that were physiologically balanced for rats, in which about 25% of the solids consisted of lactose. The same kinds and level of fats were used in the four modified products as those used in the first experiment. In the third experiment, the modified products contained nutrients that were physiologically balanced for infants. All four products contained the same level of fat as used in the first two experiments. Three of the products contained fat mixtures that varied in content of milk fat and cocoanut oil and the fourth product contained milk fat alone. Multiple levels of vitamin B_6 were used in the products of all three experiments.

M46. Market agency education assists dairy operators in solving engineering and cost problems. A. L. RIPPEN, Michigan State University, East Lansing.

A program in market agency education has been introduced for dairy plant operations in Michigan. Some of the services offered include assistance to improve operating efficiency through consultation on dairy engineering problems and suggested plant layouts. Studies

have indicated wide cost variations between plants in various phases of processing. Some examples of variations in costs of processing steps for 100 lb. of milk are: *(1)* Homogenization costs of 1.3, 5.5, and 10.5¢ for plants processing 80,000, 10,000, and 5,000 lb. per day, respectively. *(2)* Bottle washing costs for 100-bottles-per-minute capacity of from 9 to 16.0¢ for plants handling 100,000 and 20,000 lb. per day, respectively. Suggestions for reducing operating expenses often include more efficient plant layout, improved utilization of labor and equipment, and better processing methods. The trend of plant consolidations will likely continue as a means of increasing volume, with a greater opportunity to reduce units operating costs. Dairy plant operators have responded favorably to the program.

M47. Influence of rapid cooling on the development of a rancid flavor in milk produced by bulk systems. P. E. JOHNSON AND R. L. VON GUNTEN, Oklahoma State University, Stillwater.

The influence of rapid cooling of milk on the activation of lipase was suspected while studying other phases of the rancid-flavor problem in bulk-handling systems. Composite samples of individual cow's milk which had been collected at the discharge end of a pipeline system and allowed to set at milk-room temperature during the milking operation had an average fat acid degree value of 1.63; whereas, corresponding tank samples collected at the end of the milking period had an average fat acid degree value of 3.24. To further investigate the effects of rapid cooling, samples of milk from individual cows were collected at the discharge of a pipeline system and divided into two portions. One portion was cooled immediately, and cooling was delayed on the other portion. At the end of approximately 48 hr., the average fat acid value of the slowly cooled samples was 1.65, whereas on the rapidly cooled samples it was 2.70. In farm operations where rancid flavor was a problem, on alternate pickup days the refrigeration on the tank was not turned on for approximately 1 hr. after the milking began. On the other alternate pickup days, normal cooling was practiced. The average fat acid value on the milk from these farms was 1.69 when the cooling was delayed. Under normal cooling operation the average fat acid value was 2.36.

M48. A radiometric method for measuring stainless steel corrosion. W. J. HARPER, The Ohio State University, Columbus.

Under acidic conditions, radioactive phosphorus will react irreversibly with the surface of stainless steel. This reaction has been utilized to provide a basis for a rapid quantitative method for measuring stainless steel corrosion. Two to three microcuries of P^{32} in $N/1$ H_3PO_4 were applied to 24 $cm.^2$ of the surface of 2- by 3-in test strips of stainless steel. The strips were dried at 100° C. for 30 min. The unreacted P^{32} was removed by a standardized washing procedure and redried at 100° C. for 30 min. The strips then were totally immersed in the corrosive test solution at room temperature and after 12 hr. the amount of P^{32} removed from the stainless steel was measured. The concentration of P^{32} provided an index of corrosion. Sodium chloride and various cleaners and sanitizers were used as test solutions with stainless steels of the 200, 300, and 400 series. The data obtained were in relative agreement with published results, using other methods of measuring corrosion. Comparison was made between the radiometric method and the loss of weight after total immersion for 60 days at room temperature. The results of the two methods agreed in magnitude with various test solutions.

M49. Factors of value design associated with the homogenization of milk.* J. J. BETSCHER, W. J. HARPER, I. A. GOULD, AND D. A. SEIBERLING, The Ohio State University, Columbus.

An experimental homogenizer valve assembly was designed and constructed to permit variation of some of the factors associated with homogenization. The time required for a particle to pass through the valve clearance, the velocity through the clearance, the entrance and exit conditions of the valve, and the effect of two or more valves in series were studied. Valve performance was evaluated in terms of both operating characteristics and homogenization efficiency. The data indicated that the efficiency of homogenization was directly related to the velocity through the valve clearance. Neither the efficiency of homogenization nor pressure varied with valve length. With a valve which was modified by beveling the internal diameter at one of the ends, the pressure drop and homogenization efficiency were affected when the beveled end was used as the valve entrance. However, when the beveled end was used as the valve exit, only the efficiency of homogenization was affected. In multiple stage valves the efficiency of homogenization appeared to be more closely related to the total pressure drop than to the number of valves in series.

* Supported in part by funds from the Ohio Dairy Products Research Fund.

M50. Influence of bias in different age groups on consumer preferences of chocolate drink. H. S. WILLARD AND W. R. THOMAS, University of Wyoming, Laramie.

To find out if age of the consumer alters the bias produced by the serving order of chocolate drink, between 20 and 30 participants of both sexes in each of the fourth, fifth, and sixth school grades, and the same number of students of college age, were given three samples successively of chocolate drink per session, and

were asked to express the degree of likes and dislikes on a Hedonic scale. Each drink was identical except for the brand of cocoa. Fourteen different brands were used, with one brand of cocoa as control appearing at each session, the two other brands being different. Orders of serving were altered in the following ways: *(1)* Same order in all grades, *(2)* order reversed in all grades, *(3)* order changed between each grade, and *(4)* order changed within each grade. The average scores declined with the order of serving in each grade, but with lesser degree from the fourth to the college grade. Statistical treatment of the data by determining the least significant differences indicated that differences between the first and third serving were statistically significant at the 5% level in the fourth, fifth, and sixth grades, but not at the college level.

M51. Production of a high-nitrogen, low-lactose product from whey using an automatic pH control system. D. R. Arnott, Purdue University, Lafayette, Indiana, and S. Patton and E. M. Kesler, The Pennsylvania State University, University Park.

A product with increased nitrogen content has been produced from fermented Cheddar cheese whey treated with anhydrous ammonia. The ammonia was added continuously with an automatic pH control device. The pH of the whey was lowered to approximately 5.0 with *Lactobacillus bulgaricus*. This pH level was maintained throughout the fermentation period. Nitrogen levels in excess of 0.80% were achieved in less than 15 hr. of fermentation with a 10% inoculum. The lactose content was lowered to less than 1.0%. The ammoniated whey was concentrated to approximately 40, 50, 60, 80, and 90% total solids. Analyses of the concentrated whey included fat, lactose, ash, and lactic acid. Nitrogen loss durnig concentration ranged from 4.33 to 8.93% of the total nitrogen present. The concentrated whey stored at room temperature for 153 and 365 days showed a maximum nitrogen loss of 1.0% for the 365-day period. This product may have value as a ruminant feed, as a fertilizer, or as a fermentation additive.

M52. The manufacture of improved quality sherbets. W. S. Arbuckle and E. A. Day, University of Maryland, College Park.

A survey of commercial sherbets indicated a general lack of quality when compared to the ice cream marketed by the same manufacturers. Consequently, investigations were conducted to determine the effect of composition on the freezing point, handling properties, body and texture, total acidity, and consumer preference. The physical properties of the sherbets were controlled primarily by both the quantity and the type of sweetener used, along with a constant amount of stabilizer. Sweeteners used were sucrose, dextrose, and three corn syrup solids (C.S.S.) preparations with dextrose equivalents (D.E.) of 30, 42, and 54. The low conversion (30 D.E.) C.S.S. was most satisfactory when used with sucrose (1:1) to a total of 26% sweetener solids, for obtaining sherbets with a smooth texture and chewy body. This sherbet had a freezing point of 28° F. and exhibited superior handling properties. The other C.S.S. preparations were required in higher concentrations to achieve the same effect on body and texture, but resulted in a substantial lowering of the freezing point. A total acidity of from 0.55 to 0.60% was most desirable in practically all sherbets studied. The significance of variation of composition on caloric values and the results of consumer preference tests will be discussed.

M53. A centrifugal ultrafiltration method for studying the soluble constituents of ice cream. B. W. Tharp and P. G. Keeney, The Pennsylvania State University, University Park.

Ultrafiltration of ice cream through cellulose-dialyzing membranes in a refrigerated centrifuge at temperatures above 14° F. gave sufficient yield of ultrafiltrate for analysis. To determine if ultrafiltrates obtained by this method were representative of the unfrozen portion of the ice cream, comparisons were made, using ultrafiltrates of ice cream and ice cream mix, at 21 to 28° F. Calcium content, freezing point, and refractive index were used as the basis for these comparisons. The calcium content and refractive index of ice cream ultrafiltrates increased, and freezing point decreased, as temperatures of ultrafiltration were lowered. The magnitude of these changes was in accordance with accepted theories concerning concentration of soluble constituents in the unfrozen portion of ice cream. Using the data collected in this study, calculated values for the amount of water frozen at various temperatures closely agree with theory, as well as with values obtained at similar temperatures by another investigator. Therefore, these ultrafiltrates are representative of the unfrozen portion of ice cream.

M54. Influence of selected ingredients on the melt-down of vanilla ice cream. P. J. Roberts and D. R. Frazeur, Purdue University, Lafayette, Indiana.

Experiments designed to determine the influence of certain ice cream ingredients on the melt-down of vanilla ice cream were conducted, using standardized methods of mix preparation, freezing, holding, and melting. A standard mix composed of 12% milk fat, 11% milk solids-not-fat, and 15% sucrose was used wherever applicable. Stabilizers and emulsifiers were used at manufacturers' recommended levels. Satisfactory ice cream melt-downs were obtained with sodium alginate, Irish moss extract, and car-

boxymethyl cellulose either with or without emulsifiers. Guar seed gum, locust bean gum, gum karaya, and gelatin did not yield satisfactory melt-downs unless used with emulsifiers. It was found that in certain cases, the curdiness caused by a particular stabilizer could be improved by one emulsifier, but another emulsifier migh have little or no such action. The degree of curdiness observed was inversely related to the rate of melt-down. The addition of 0.15% disodium phosphate or sodium citrate had less effect on melt-down than did the optimum emulsifier. The substitution of corn syrup solids for part of the sucrose caused curdiness in proportion to the amount added.

M55. Interrelationships of heat treatment, solids-not-fat, and calcium chloride to the yield of cottage cheese. C. W. Dill, W. M. Roberts, and R. J. Monroe, North Carolina State College, Raleigh.

The effects of pasteurization temperature, solids-not-fat content, and the addition of varying amounts of calcium chloride to milk after pasteurization on the yield of cottage cheese were studied. All three variables affected the yield of cottage cheese expressed as pounds of cheese per pound of solids-not-fat in the skimmilk. Fortification of the skimmilk with nonfat dry milk to a solids-not-fat content of from 10 to 11% increased the yield of cottage cheese per pound of solids-not-fat. Increases in pasteurization temperature resulted in progressive increases in the yield of cottage cheese over the entire temperature range studied. The addition of varying amounts of calcium chloride to pasteurized skimmilk affected the yield of cottage cheese only slightly when considered separately, the main effect from calcium chloride being its desirability in the presence of increased heat treatment and/or increased solids-not-fat content. There were significant interactions between the three variables studied.

M56. Effect of skimmilk heat treatments on cottage cheese manufacture. D. B. Emmons, A. M. Swanson, and W. V. Price, University of Wisconsin, Madison.

Defects in cottage cheese from nonfat dry milk have been attributed to overheating skimmilk during processing. Defects in conventionally manufactured cottage cheese occur when skimmilk is heated sufficiently to alter the whey proteins. When skimmilk is heated to 175° F. for 30 min., the combined use of 20 ml. of rennet per 1,000 lb. of skimmilk and of the A-C test restores the moisture-expelling properties of the curd and minimizes fragility. Recovery of skimmilk solids as curd is increased approximately 10%. Critical judges find a certain amount of mealiness and unevenness in body when the curd is cooked and washed conventionally. The pH of the salted and creamed curd needs adjustment. Mealy, pasty, soft, and fragile defects occur in curd made by conventional methods from skimmilk that is overheated while concentrated (e.g., 47% total solids, 160° F. for 15 min.). No detectable changes in whey proteins, A.D.S.A. (pepsin:HCl) curd tension, or solubility index need occur. Rennet curd strength measurements on the reconstituted product and a modification of this test using calcium chloride reflect the severity of the heat treatment given to the concentrated skimmilk.

M57. Determination of the curd-making properties of cottage cheese type nonfat dry milk. W. K. Stone and P. M. Large, Virginia Agricultural Experiment Station, Blacksburg.

Cottage curd was made from an unknown powder in one laboratory vat and from a control powder in a similar vat. The starter and cheesemilk in each vat ewer made from the respective powder being used. Seven pounds of starter were thoroughly mixed with 25.0 lb. of freshly reconstituted 11.0% milk at 95° F., and 0.08 ml. of rennet extract added. When the pH of the curd was 4.70, it was cut into ¾-in. cubes, the whey was increased to 99° F., and at pH 4.60 cooking was started. The curd was cooked at 125° F., washed, drained, packaged, and stored at 40° F. Curd quality was determined by evaluating for body and texture, and color and appearance, using the A.D.S.A. guide for scoring. Comparisons between curd made in small and large vats were made. Preliminary results demonstrate that the correlation coefficients between corresponding small and large vats for curd quality and for per cent moisture were +0.88 and +0.75, respectively; between soluble whey-protein nitrogen of powder and the corresponding curd quality, −0.37, and between rennet curd tension values of powder and the corresponding curd quality, −0.32.

M58. Effects of starters on acidity and firmness of cottage cheese coagulum. D. B. Emmons and W. V. Price, University of Wisconsin, Madison.

One lot of nonfat dry milk was reconstituted (11:89, powder:water) for experiments testing 17 commercial starters. Coagulum was formed at 90° F. with rennet and starter used at rates of 1 ml. and 70 lb., respectively, per 1,000 lb. of skimmilk. There were marked differences in relationships of titratable acidity of whey and pH of curd obtained with the different starters. The range in titratable acidity of whey at pH 5.1 was from 0.45 to 0.66%. High titratable acidities of whey at the A-C endpoint were associated with floating curd. Varied CO_2 production by starters undoubtedly explains differences in titratable acidity of whey at a given pH. Curd strength values measured with the Cherry-Burrell curd tension apparatus were closely correlated to the

decrease in pH after coagulation, regardless of the starter used. The relationship between curd strength and pH of the curd varied with the activity of the starter used; slow starters were associated with higher pH values at the time of coagulation. Starters had a definite effect on the relationship between titratable acidity of whey and curd strength.

M59. Aggregation of calcium caseinate by heat and its reversal. C. A. ZITTLE, Eastern Regional Research Laboratory, Philadelphia, Pennsylvania.

Solutions of calcium caseinate stable at 25° C. precipitate when heated to 90° C. and redissolve at the lower temperature. This reversible aggregation has been studied further by using more dilute $CaCl_2$ solutions, so that the aggregation (turbidity) can be measured in a spectrophotometer. One per cent solutions of casein at pH 6.3 were studied, containing 4 to 6 mm. of $CaCl_2$ per liter. Turbidities were estimated from the light extinction at 600 $m\mu$ in a cell at 30°. Solutions were raised from 50 to 90°, held there for various periods of time, then returned to 30°, and the decrease in turbidity followed with time. This aggregation-time reversal curve was compared with the curve obtained when the solution was placed at 30° without prior heating. Whole casein, α-casein, paracasein, and casein with sodium phosphate were studied in detail. The aggregation of whole casein at elevated temperature reversed quickly at 30° and then followed a curve above the 30°-no heat curve, the distance above the 30°-no heat curve was determined by the temperature and duration of heating. Detailed results for this and the other caseins studied will be reported.

M60. Separation of a calcium-soluble fraction of casein from isoelectric casein. K. K. FOX, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The presence of a calcium-soluble casein fraction in the caseinate complex of milk is of interest, in that its concentration and mode of association with the other constituents of casein may govern the stability of this complex during heating and storage of concentrated milk products. A method for its isolation from isoelectric casein, requiring only equipment normally found in the usual dairy laboratory, is presented. A 2% solution of sodium caseinate at pH 11.0 and 5° C. is made 0.15 *M* with $CaCl_2$. The pH is lowered to 8.3 with *N*/10 HCl and the fraction aggregated by calcium is removed. The supernatant is warmed to 30° C. and passed through a milk clarifier. The clarified liquid is brought to pH 4.7 and the resulting precipitate washed to remove calcium. The precipitate contains two components, the principal component sedimenting at about 11.8 S (Svedberg units) and the lesser component at 1.35 S. The lesser component is soluble, and the main component not soluble, in 6.6 *M* urea. The isolated principal component has a relatively constant S (about 11.8) over the pH range 7.8–9.8, dissociating to exhibit lower values at pH 10.3 and above.

M61. Additional evidence of a stable complex between β-lactoglobulin and α-casein. J. C. TRAUTMAN AND A. M. SWANSON, University of Wisconsin, Madison.

Electrophoretic analyses (phosphate buffer, pH 6.8, ionic strength 0.1) were made on proteins from raw skimmilks, skimmilks that had been heated to 180° F. for 30 min., and skimmilks containing sulfhydryl-blocking agents before receiving a similar heat treatment. In the heated skimmilks, at least two-thirds of the original β-lactoglobulin disappeared, but in the skimmilks containing the —SH blocking compounds the electrophoretic patterns were comparable to the original raw skimmilks. Similar results were obtained with various isolated casein fractions. These results indicate a stable complex and that the —SH groups may be involved in its mechanism.

M62. Electrophoretic and ultracentrifugal analysis of casein complex size fractions from milk. F. A. HECKMAN, W. L. CHOATE, AND T. F. FORD, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Conflicting reports [*(1)* Hostettler, Von H., *et al., Landw. Jarhb. Schweiz,* **63**: 31-67 (1949); *(2)* Heyndrickx, G. V., and Vleeschauwer, A. De. *Experientia,* **8**: 317-320 (1952)] on the relative concentrations of α- and β-casein in fractions of the caseinate complex separated by differential centrifugation led to a reinvestigation of this system. Fractions were separated and dialyzed against veronal buffer for electrophoretic and ultracentrifugal study. It was found that the ratio of α- to β-casein is essentially constant over a wide range of sizes, in agreement with *(1)*. One component with an S value of 3.5 was predominant in all fractions. The depleted supernatant solutions showed varying ratios of α- to β-casein when prepared for electrophoresis in the same manner, in agreement with *(2)*. It is concluded that the presence of serum proteins in the dialyzed preparation of the supernatant liquid results in changes of interaction between α- and β-casein. The constancy of composition of the centrifugally separated complex is considered to be strong evidence that the particles of various sizes in milk have an essentially constant composition.

M63. Voluminosity of caseinate complex in milk, and reconstituted sediments. C. H. WHITNAH AND W. D. RUTZ, Kansas State College, Manhattan.

Six liquids ranging from 0.444 to 0.162% N were prepared from a sample of milk using *(1)* a commercial cream separator, *(2)* a Sharples supercentrifuge, and *(3)* a Ford "bowl rotor." Sediment from the Sharples bowl was reconstituted with water to contain 0.819% N. Half of this suspension was diluted with an equal weight of water. Such dilutions were repeated until six concentrations of sediment were obtained. Viscosities were measured at 15 temperatures from 4 to 70° C. Fluidities (ϕ) were calculated relative to *(1)* the fraction containing least N, and *(2)* to water. The slope of each series of five or six pairs of values for ϕ and concentration was determined by least squares and converted to voluminosity, using the equation $V = 0.4\ d\ (\phi/\phi)/d$ concentration. Approximate average voluminosities ranged from 6.0 ml/g at 4° C. to 4.8 at 20° C. and 2.8 at 70° C. when ϕ' was the fraction containing least N. Corresponding values when ϕ' was water were 4.5, 3.5, and 2.0 ml/g. Irregularities in plots of V vs. t were larger than can be explained as errors of measurement.

M64. Size memory of casein colloid particles. W. L. Choate, F. A. Heckman, and T. F. Ford, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Fractions of the caseinate complex, prepared by differential centrifugation, followed by dialysis of each fraction against veronal buffer, have been shown to have the same ratio of α- to β-casein by elesctrophoretic analysis. Ultracentrifugal analysis of thees solutions showed one major component with an S value of 3.5 to be present. These preparations when redialyzed against skimmilk become opaque and resemble milk. Ultracentrifugal analysis shows S values in the same range as in the original milk. The fractions with larger S values in milk have larger S values in the redialyzed colloids, ranging from 100 to about 1,000.

M65. Effects of accelerated electron irradiation on the casein of skimmed milk and sodium caseinate sols. J. R. Brunner and R. C. Nickolas, Michigan State University, East Lansing.

The cold irradiation of a casein sol with accelerated electrons changes the multi-component characteristics of the electrophoretic pattern of sodium caseinate to a pattern showing a single component; the μ of which corresponds to the μ of the α-casein component. Ultracentrifugal diagrams of these irradiated caseins show but one sedimentation boundary. These observations are strongly indicative of a complex formation between α- and β-casein. Irradiation levels required to produce the single-peak phenomena are approximately 1×10^6 and 4×10^6 reps for sodium caseinate sol and the casein of skimmed milk, respectively. Other observations showed an increase in the rennet coagulation time, lower curd tension, and lower heat stability at 120° C. as a result of irradiation. No appreciable change in pH or NPN content was noted. Titrations in aqueous, alcohol, and acetone media did not indicate a cleavage either in the peptide linkage or in the phosphobond. Fresh milk plasma dialysate and/or certain of its constituent components exhibited a sparing action on the irradiated casein.

M66. Effects of proteins on agglutination of fat globules. Alan Kenyon and Robert Jenness, University of Minnesota, St. Paul.

Some of the individual components responsible for agglutination of fat globules were investigated. The techniques employed to assess agglutination included visual and microscopic examination, cream layer formation, and fat content of gravity skimmilk. In any one series of tests the source of fat globules was a single lot of cream which was reconstituted with skimmilk and any individual constitutents desired. Either homogenization or heat treatment of skimmilk prevents agglutination of fat globules, but a mixture of equal parts of homogenized and heated skimmilk produces normal creaming, which is in agreement with the report of Samuelsson and coworkers in Sweden. Addition of 15 γ of colostral euglobulin per 100 ml. restored the creaming ability of heated but not that of homogenized skimmilk. On the other hand, β-lactoglobulin, α-lactalbumin, pseudoglobulin, and blood serum albumin, when added to heated skimmilk in the amounts in which they usually occur, had no effect. An attempt was made to identify the factor inactivated by homogenization. Electrophoretic analysis of protein adsorbed on cold fat globules and released upon warming revealed the presence of a component with a motility of -4.5×10^{-5} cm.^{2} volt^{-1} sec^{-1} at pH 8.6, $\mu = 0.1$ in veronal buffer.

M67. A comparative study of the influence of heat, concentration, and rennin action on the sedimentation of proteins in milk. I. S. Verma and C. W. Gehrke, University of Missouri, Columbia.

It was found earlier that the enzymic stage of renning action in milk caused increased association of casein molecules. Further studies were conducted to determine the effect of added Ca- and P-salts. The simultaneous addition of Ca and rennet showed a very significant increase in sedimentation, and the composite effect was more than the additive effects at 8° C., where only the first phase of rennin action occurs, showed an increased sedimentation of N of 64.4 mg. % as compared to the control; whereas, addition of Ca caused an increase of 36.4 mg. % of N. Rennet and Ca addition together increased the sedimentation of N by 173.4 mg. %. The results have been further confirmed by studying milk treated with ion-exchange resins

or oxalate to eliminate the Ca effect. Increased sedimentation of proteins is demonstrated to be the result of establishment of additional intermolecular Ca-bridges on foci created by rennin action on casein. Comparative studies were made on milk concentrated by heat and freeze-drying. The results reveal qualitative similarities but are not significant.

M68. Phospholipid and casein interactions. S. M. HUSAINI AND A. M. SWANSON, University of Wisconsin, Madison.

Complex formation between casein and milk phospholipids during heating of simple systems was studied. Phospholipids were prepared from dry buttermilk solids by extraction with 1:1 ethanol-ethyl ether. Casein was prepared by isoelectric precipitation from fresh skimmilk. In most of the work, solutions were prepared by mixing 3% casein and 1% phospholipids in phosphate buffer (pH 6.8, 0.1 ionic strength) at 3–5° F. These solutions were heated in an oil bath at 180° F. for 15 min. and cooled immediately. The following methods were used as a means of determining complex formation (interaction): nonavailability of part of the phospholipids to ethyl ether extraction, changes in isoelectric point of casein, nephelometry, and viscosity measurements. Using phosphorus determinations as a means of measuring available phospholipids after ethyl ether extraction, it was found that 63.0% of the phosphorus could not be recovered from the casein-phospholipid mixtures after heating. In casein-phospholipid mixtures of very low concentration there was an increase in haze value after heating. A change in the isoelectric point of the casein, and a decrease in viscosity as measured by the Ostwald pipette method, were also observed when the casein-phospholipid mixtures were heated. Similar results were obtained when α-casein was used. These changes would indicate that an interaction took place during heating.

M69. Optical ultracentrifugal resolution of casein complex particles in milk. T. F. FORD, LORRAINE W. KLIPP, G. A. RAMSDELL, AND W. L. CHOATE, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The air-driven, air-floated capillary tube ultracentrifuge has been used for the study of the casein colloids of whole milk and skimmilk. Various optical techniques have been employed. It has been found that many definite sizes of casein complex particles can be sharply resolved with whole milk, but only a few dominant sizes can be identified with skimmilk. It is suggested that whole milk contains a size-stabilizing substance; that the persistent dominant sizes in skimmilk are determined primarily by physical forces. Sedimentation constants are tabulated.

M70. Application of tritium and carbon^{14}-labeled compounds in cheese ripening. A. WOLIN AND F. V. KOSIKOWSKI, Cornell University, Ithaca, New York.

Milk components were labeled with tritium or carbon,^{14} so as to follow labeled breakdown products of ripening cheese. Randomly tritiated casein was prepared by the Wilzback technique (*J. Am. Chem. Soc.*, **79**: 1013. 1956). Five grams of casein were incubated with tritium gas (H^3) for 72 hr. at 27° C. This tritiated casein which was washed to remove the labile H^3 attained a specific activity of 6 mc. per gram. Due to the low beta energy of tritium (0.0189 Mev), radioautography and windowless–gas flow counting were employed. The windowless counter is more sensitive for C^{14} than the thinnest mica window counter and it also enables the sensing of H^3. A rate of 25 counts per minute was obtained on one sample of labelled C^{14} casein with a thin mica window counter, in contrast to 250 counts per minute with the windowless counter. Labeling of compounds with tritium is less expensive than C^{14}, and a higher specific activity is achieved. The C^{14}-labeled casein displayed 125 c.p.m./0.1 mg., whereas the H^3 casein was 94 c.p.m/6.9×10^{-6} mg. Another advantage is that milk can be directly tagged with tritium, whereas C^{14} must be fed or injected into the cow to label the milk components.

M71. Characteristic Cheddar cheese flavor in relation to hydrogen sulfide and free fatty acids.[*] T. KRISTOFFERSEN AND I. A. GOULD, The Ohio State University, Columbus.

Fourteen lots of commercial Cheddar cheese were cured under controlled conditions for 1 yr. The cheese was graded and analyzed for hydrogen sulfide and free fatty acids at definite intervals. The rate of development of characteristic Cheddar flavor (C.F.) was greater in raw- than in pasteurized-milk cheese. The C.F. of high-quality, pasteurized-milk Cheddar cheese was considered equal to that of raw-milk cheese after about 6 mo. of curing. Pasteurized-milk cheese, consistently graded B or below, usually was rated lower in C.F. than either the raw-milk cheese or the pasteurized-milk cheese of high quality. Intensity of C.F. appeared to be related to the molar ratios of both 0_2 and longer-chain fatty acids and acetic acid to hydrogen sulfide. Cheese with ratios of $C_6+/H_2S \times 100$ from 1.5 to 5.0 and $C_2/H_2S \times 100$ from 4 to 9 were rated highest in C.F. Ratios outside these ranges were associated with lower C.F. intensities. The concentrations of hydrogen sulfide in the cheese fluctuated during ripening, whereas those of free fatty acids generally showed a continuous increase.

[*] Supported in part by funds from the American Dairy Association and the Ohio Dairy Products Research Fund.

M72. Identification of volatile carbonyls from Cheddar cheese. E. A. Day and Mark Keeney, University of Maryland, College Park.

Forty pounds of high-quality, rindless, 16-mo.-old Cheddar cheese was macerated, dispersed in water, and distilled at from 25 to 35 mm. Hg pressure. Evolved volatiles were condensed in cold traps, reacted with an acidic 2,4-dinitrophenylhydrazine solution and the 2,4-dinitrophenylhydrazones (2,4-DNPH) were obtained by chloroform extraction. After removal of the chloroform, the 2,4-DNPH mixture was exhaustively extracted with hexane and the hexane extract was condensed and resolved on nitromethane-hexane-Celite partition columns. All solvents were carefully treated prior to use, to avoid artifacts. Identification of the 2,4-DNPH's was based on: *(1)* comparison of threshold volumes of knowns and unknowns on the partition column; *(2)* melting points and mixed melting points; *(3)* absorption spectra in the 220 to 400 mμ region; and *(4)* regeneration of the 2,4-DNPH for characterization of odor properties. The 2,4-DNPH's of 3-methylthiopropanal (methional), formaldehyde, acetaldehyde, acetone, butanone, 2-pentanone, 3-methylbutanal, 2-heptanone, 2-nonanone, acetoin, and diacetyl were identified. The 2,4-DNPH's of 2-tridecanone and 2-undecanone have been tentatively identified by techniques *(1)*, *(3)*, and *(4)* above. Of the carbonyls identified, 3-methylthiopropanal appears to be most noteworthy in Cheddar flavor. A number of the volatile carbonyls have yet to be identified.

M73. Methyl ethyl ketone and other carbonyl compounds in Cheddar cheese. R. Scarpellino and F. V. Kosikowski, Cornell University, Ithaca, N. Y.

Cheddar cheeses at different stages of ripening were obtained from several manufacturers and the flavor intensity of each sample was assigned a place on a numerical scale. Total carbonyl content of the cheeses was determined by colorimetric analysis of the 2,4-dinitrophenylhydrazone derivatives, and the individual carbonyl compounds were separated through paper chromatography. A relationship between flavor intensity and the quantitative and qualitative nature of the carbonyl compounds was tentatively indicated. Among the carbonyl compounds observed were three compounds hitherto unreported in Cheddar cheese chemistry literature. Their chromatographic positions indicated chain lengths of four, six, and seven carbons. The four-carbon compound, identified by its melting point as methyl ethyl ketone, existed at relatively high concentrations in all the aged cheeses. More research is being conducted to establish the origin of these compounds and their contribution to flavor.

M74. Further studies on Cheddar cheese making by the Australian short method. P. A. Downs, University of Nebraska, Lincoln.

Since our first report in 1955, twelve, 300-lb. lots of cheese have been produced by this method, resulting in a slow-curing, good-quality cheese. The slow curing seemed to be related to the lack of acid development in the curd during cheddaring. In an effort to correct this, the originators of the method have recommended the use of *Streptococcus lactis* with *Streptococcus thermophilus* in place of *Streptococcus durans*. This was found to be an improvement. A modified schedule using *S. thermophilus* alone, and suggested later by Australian workers, has been tried without improvement owing to the slow acid development in the curd. The lots of cheese made by the short method over 3 yr. have all cured to a high-quality cheese. The original modified method is considered as a practical, time-saving commercial method of making Cheddar cheese, which tends to cure slowly.

M75. Effect of salt on acid development in Cheddar cheese. H. E. Walter, A. M. Sadler, C. D. Mitchell, and R. E. Hargrove, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Great differences encountered in acid development by single strains of *Streptococcus lactis* and *Streptococcus cremoris* in Cheddar-cheese curd after salting led to this study. For each of 20 experiments, one lot of curd was divided after milling into six equal portions, and varied amounts of salt were added to yield from 1.0 to 2.25% in the cheese. Single strains of *S. lactis* and *S. cremoris*, and mixtures of both species, were used as starters. In general, acid production by most single strains of *S. lactis* was not inhibited by less than 1.6% salt and not significantly by from 1.6 to 2.0%; whereas, most single strains of *S. cremoris* were inhibited slightly by 1.4%, definitely by 1.6%, and almost completely by 2.0%. Some strains of *S. cremoris* were more salt-tolerant than some strains of *S. lactis*. Mixtures of *S. lactis* and *S. cremoris* were much more uniform in salt resistance than single strains of either species. They were not inhibited by less than about 1.5% and not significantly by concentrations between 1.5 and 1.9%. Acid bitterness decreased with increased salt. Fruity flavors usually accompanied insufficient acid development. Cheese containing 2% salt failed to ripen normally.

M76. Acidity and age of natural cheese as factors affecting the body of pasteurized process cheese spread. N. F. Olson, D. G. Vakaleris, W. V. Price, and S. G. Knight, University of Wisconsin, Madison.

Two varieties of cheese, Cheddar and Dariworld, were made with normal and high pH.

They were aged at 50° F. and at various intervals were individually used as the base for cheese spreads of constant composition. The acceptability of the spreads was measured organoleptically, by penetrometer and by melting tests. It was found that natural cheese with high pH was undesirably firm in body at every age, and this firmness persisted in the spreads made from such cheese. All tests showed that the age of the cheese when processed into spread affected acceptability. Improvements were found as the cheese aged to 60 days. After that, penetrometer values tended to show increased hardness and melting tests continued to improve with age. Storage of spreads at 50° F. was possible without significant changes in penetrometer values for at least 90 days, but deterioration was measurable after 15 days of storage at 90° F. and had reached a critical level for commercial use in approximately 30 days. The normal Dariworld cheese appeared to be superior to normal Cheddar cheese for making this type of spread. It reached desirable processing characteristics at an earlier age and the spread made from it was more durable in storage.

M77. Interrelationships between pH, populations of Propionibacterium shermanii, levels of free fatty acids, and the flavor ratings of Swiss cheeses. F. E. Kurtz, J. A. Hupfer, E. A. Corbin, R. E. Hargrove, and H. E. Walter, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Published correlations between fatty acid levels and flavor of Swiss cheese have been sufficiently positive to prompt further study. Fifteen experimental cheeses were examined periodically during 12 mo. for fatty acid levels, pH, bacterial population, and flavor; samples of five commercial cheeses were rated on flavor and free fatty acid content only. Variations in pH of the cheeses off the press from 4.95 to 5.37 were experimentally induced. The pH showed a general correlation with the maximum populations of *Propionibacterium shermanii* which ranged, with increasing pH, from 145 million to 2,420 million organisms per gram of cheese. The maximum content of acids ranged as follows: acetic, 38–59; propionic, 50–104; butyric, 6–40, and the higher acids, as a group, 22–48 μM per gram of cheese. There was a correlation between the maxima of propionic acid and *P. shermanii*. The data indicate that the nutty, but not the sweet, flavor may be related to the propionic acid level. It appears that propionic acid is a component of the flavor complex but that other compounds are responsible for both the nutty and the sweet characteristics of typical Swiss cheese flavor.

M78. Continuous, mechanical process for draining and hooping Blue cheese curd. D. M. Graham, Clemson Agricultural College, Clemson, S. C.

A mixture of curd and whey is pumped from the cheese vat into a rotating, cylindrical sieve. After removing the free whey, the curd passes continuously onto an inclined, vibrating chute. A 40-mesh wire sieve mounted on the vibrating chute lightly dusts the curd with dry mold powder immediately before the curd falls into the hoops. Blue cheese curd has been pumped without damage to particle structure by either an Anderson Model 520 or a Waukesha Model DO curd pump at flow rates up to 20 gal. per minute through 1½-in. stainless steel tubing. Some curd damage resulted from use of a standard centrifugal milk pump. The 7½-in. diameter sieve rotated at 20 r.p.m. and was inclined 15 degrees from horizontal. Hoops were filled without hand manipulation of the curd at the rate of two to three per minute. The cheese was set at 90° F. in the usual manner, cut into ½-in. cubes, and pumping started when the whey acidity was 0.16%. Moisture content of four wheels selected at random from a 300-gal. vat ranged from 44.47 to 45.24% and fat content ranged from 30.0 to 30.5%. Mold scores after 3 wk. of aging averaged one to two points higher for machine-hooped than for manual-hooped cheese. Distribution of veins was markedly improved, probably owing to a more uniform inoculation and open texture in the machine-hooped cheese.

M79. Selection of Penicillium strains for Blue cheese. D. M. Graham, Clemson Agricultural College, Clemson, S. C.

Eight strains of *Penicillium*, three from laboratory collections and five isolated from cheese, were studied. Criteria for evaluating the strains were *(1)* organoleptic scores of cheese inoculated with each mold, *(2)* volatile acidity, per cent nonprotein nitrogen, and neutral carbonyl compounds in the cheese, and *(3)* ability to produce neutral carbonyl compounds in laboratory cultures. Statistical treatment of the organoleptic scores of 14 vats of cheese ranked the strains in five groups: *(1)* P12, P108, M; *(2)* R, *(3)* I, C; *(4)* B, and *(5)* N. Under ideal curing conditions, strains M and R produced excellent flavor, but in routine manufacture frequently they failed to develop adequate veining. Addition of 0.2 to 0.4 γ $FeCl_3$ per 100 g. milk did not improve growth of strains M and R. Strains I, P12, and P108 consistently produced well-veined and good-flavored cheese in routine manufacture, under a variety of conditions in natural cave and mechanically refrigerated curing. Inoculation with two, three, or four strains in 15 different combinations produced less desirable cheese than single-strain inoculation. Correlation between flavor scores, volatile acidity, and neutral carbonyl compounds was fair.

M80. Development of a domestic Feta cheese. C. Efthymiou and J. F. Mattick, University of Maryland, College Park.

Cheeses were made from pasteurized and raw milk. The lipolytic agents included in the study were natural milk lipase and Dairyland Food Laboratories' "pregastric-esterases"—Capalases K, L, KL, and Italase C. The bacterial cultures used, pure and in mixtures, were *Streptococcus lactis, Lactobacillus acidophilus,* and *Lactobacillus casei.* The milk was ripened with 1% starter at 86° F. until 0.025% acid developed. Commercial lipases were added at a rate of 1.5 g/100 lb. milk. Milk was coagulated with liquid rennet at 86° F. in from 40 to 60 min. Curd was cut with 1-in. knives, stirred with mild agitation at 86° F. for 20 min., then dipped into perforated 12-in.-cube cheese forms. After sufficient drainage, the curd was removed from the forms, cut into 4-in. squares, and placed in a curing room at 65° F. and 85% relative humidity. The cheeses were dry-salted at a rate of 3% of the weight of the cheese. After from six to eight days of curing, the cheeses were washed, placed in sealed cans with sufficient 3% NaCl solution to fill the cans, and stored at 40° F. Feta cheese with excellent flavor and body and texture characteristics was developed using pasteurized milk, Capalase KL, and a mixed culture of *S. lactis* and *L. casei.*

M81. The vitamin B_6 potency of cheese. I. L. Hathaway, University of Nebraska, Lincoln.

The vitamin B_6 potency of 406 samples of 31 cheeses was determined by microbiological methods. The cheeses included in this study were American, Beer Kaese, Blue, Brick, Camembert, Caraway, Wisconsin Cheddar, New York Cheddar, Cheese spread, Cottage, Dry Curd Cottage, Cream Cheese, Philadelphia Cream, Edam, Garlic, Gouda, Gruyère, Kaukauna Klub, Liederkranz, Limburger, Mozzarella, Muenster, Parmesan, Pepper and Bacon Cheese, Port Salut, Provolone, Roquefort, Swiss, and Velveeta. These samples were obtained from the Nebraska Experiment Station Creamery, from cheese factories and stores in Lincoln and Omaha, Nebraska, or from Milwaukee, Wisconsin. Since the samples were representative of the cheeses which are available in most markets, the results are believed to indicate the vitamin B_6 value of this product. The B_6 values ranged from 44 γ per 100 g. of dry curd cottage cheese (19 samples) to 251 γ per 100 g. of Camembert cheese (21 samples). Of the 31 cheeses studied, Camembert, Liederkranz, and Blue cheese were the most potent in vitamin B_6.

PRODUCTION SECTION

P1. Formulation and feeding of purified diets to dairy cattle. J. H. Byers and J. R. Staubus, University of Illinois, Urbana.

Sixteen steers and one heifer were fed various purified diets and performance was measured by body weight and digestion trials. Gains followed consumption closely. Diet A containing 55% Solka-Floc, 22.8% corn starch, 10% cane sugar, 4% urea, 4% hydrogenated tallow, 4% minerals, and 0.2% A and D supplement was very unpalatable. Reducing Solka-Floc to 35% and increasing sugar to 14.8% and corn starch to 38% increased palatability and fiber digestibility. Observations and digestion trials showed that the tallow was not well digested. When corn oil replaced tallow, palatability and crude fiber digestibility were increased. The A and D supplement was replaced by purified fat-soluble vitamins without observable effect. Water-soluble vitamin supplementation individually or as a complex failed to improve gains. Inadequacies in the diets were indicated by impaired and depraved appetites, loss of condition, malfunction of joints, impaired vision, and subclinical infections at terminal slaughter. Four animals died of unknown causes. One animal showed symptoms of urea toxicity. Replacing urea with Drackett improved gains but not digestibility. Ten per cent ground cobs increased palatability, gains, and crude fiber digestibility. Work is continuing to define this effect of cobs.

P2. Effect of all-hay vs. all-silage rations on dry matter intake of lactating dairy cows; moisture and pH as factors affecting appetite. D. Hillman, C. A. Lassiter, C. F. Huffman, and C. W. Duncan, Michigan State University, East Lansing.

Two equalized groups of eight milking Holstein cows were used in a 42-day single-reversal experiment to determine the effects of an all-hay ration compared to an all-silage ration on dry matter intake, body weight, and milk production. The hay and wilted silage (25.0% D.M.) were both harvested from the same field at the same stage of maturity. Cows fed all hay averaged 3.14 lb. of dry matter per 100 lb. of body weight, gained 19.4 lb. in body weight, and averaged 28.3 lb. of milk per day. Respective values for cows fed silage were 2.46, −42.4, and 28.4. Intake and body weight changes were significantly different (1%). In a 40-day Latin-square design, trial cows were fed *(1)* regular hay, *(2)* regular silage, *(3)* hay soaked with water, 30.6% D.M., and *(4)* high pH silage, regular silage sprayed with NaOH to increase the pH to that of the hay. Milk production, gain in body weight, and dry matter intake values for regular hay were 23.4, 10.7, and 3.23; regular silage—21.6, −36.9, and 2.17; wet hay—22.4, 15.0, and 3.28, and high pH silage—21.2, −22.6, and 2.21. Differences among rations in dry matter intake and body weight changes were significant in both hay groups as compared to the silage groups, but not within each roughage.

P3. Effect of various additives on dry matter intake of heifers fed either alfalfa hay or sil-

age. J. P. Everett, Jr., C. A. Lassiter, C. F. Huffman, and C. W. Duncan, Michigan State University, East Lansing.

Twenty-four heifers averaging 674 lb. body weight were divided into three groups and offered either all hay (Group 1), wilted silage averaging 25.0% D.M. (Group 2), or lacerated-high-moisture silage averaging 17.9% D.M. (Group 3), free choice in a 4-wk. trial. All three forages were harvested from alternate areas of the same field of alfalfa in one-quarter bloom. The silages were stacked on a concrete slab with no preservative and covered with a plastic sheet. In a second trial, each group was subdivided and half of the animals fed the following additives: hay sprinkled with dilute HCl, wilted silage plus a flavor compound (Silo-joy), or high-moisture silage plus ground hay. Average daily gain and average daily dry matter intakes per 100 lb. body weight for Groups 1 through 3 were 1.69, 1.28, 0.09 lb., and 2.33, 2.01, and 1.60 lb., respectively, for Trial 1. Lowering the pH of the hay and adding Silo-joy to wilted silage had no significant effect on the dry matter intake in Trial 2. However, adding enough ground hay to the high-moisture silage to raise the dry matter content to approximately that of the wilted silage resulted in a significant ($P < 0.05$) increase in dry matter intake.

P4. Studies on forage: concentrate ratios for milk production. J. L. Morris, E. Gainer, and R. F. Davis, University of Maryland, College Park, and C. R. Richards, University of Delaware, Newark.

Six trios of dairy cows were assigned to the following rations starting 20 days prepartum and continuing throughout a 305-day lactation: *(1)* 80% of the estimated net energy (Morrison: upper level) from concentrates, 20% from hay, *(2)* 40% of the estimated net energy from concentrates, 60% from hay; *(3)* 20% of the estimated net energy from concentrates, 80% from hay. Following parturition all animals were fed ad libitum up to 110% of Morrison's recommended allowances. Beginning with the 6th wk. and until the end of lactation, the estimated net energy intake was lowered 1% of the intake of the previous week for all treatments. U. S. No. 2 leafy alfalfa and a simple grain mixture were fed throughout the experiment. Observations on the incidence of mastitis and udder edema were made periodically throughout the experiment. Digestion studies on the above rations were conducted with a change-over design with three steers. In addition, the chromic oxide indicator method was used to determine the digestibility of the rations on 13 cows. The production of 4% fat-corrected milk averaged 30.9, 27.2, and 25.1 lb. per day, with the rations ranked in decreasing proportion of concentrate. After allowing for maintenance and body weight changes, the calculated therms required per lb. of 4% F.C.M. were 0.40, 0.35, and 0.34, in the same order.

P5. Effect of level of feed intake using hay: grain ratios on feed utilization of dairy cows. C. A. Lassiter, C. F. Huffman, and C. W. Duncan, Michigan State University, East Lansing.

In a previous study, using good alfalfa hay, it was observed that as the level of feed intake increased the digestibility of hay: grain rations increased. In this study, 27 digestion trials were conducted, using nine dry cows to study this phenomenon further. A medium-grade, late-cut timothy hay and a 21.2% protein-grain mix were fed. Three hay-grain ratios were fed, 80: 20, 50: 50, and 20: 80, on a dry matter basis. Three levels of intake were used—70, 100, and 130%, where the 100% level was a typical rate of feeding dry cows averaging 23.7 lb. of feed per day. The trend toward higher coefficients of digestibility, with an increasing proportion of grain to hay, was evident with all nutrients except crude fiber. There was a definite trend toward higher digestion coefficients as the level of intake increased, although not statistically significant, except with crude protein and crude fiber. This was evident at all hay: grain ratios. The average coefficients of digestibility, regardless of hay: grain ratio, for the high, medium, and low levels of feed intake were 75.4, 70.7, and 69.4 for dry matter, 77.7, 75.4 and 71.1 for crude protein, 62.2, 50.8, and 46.4 for crude fiber, and 76.6, 72.2, and 70.3% for organic matter, respectively. This trend was most pronounced with crude fiber digestion and with the 20: 80 hay: grain ratio.

P6. Effect of supplemental fat in high-energy rations on hot-weather performance of producing dairy cows. R. R. Harris, R. E. Leighton, and I. W. Rupel, Texas A & M College, College Station.

The energy value of the low-fiber rations used in hot-weather studies (*J. Dairy Sci.*, **40**: 1391. 1957.) was increased further by increasing the fat content in the concentrates from 3.8 to 8.9%. Fat additives used were tallow and a cottonseed "foots" methyl ester product (Marcol B-75). During the summer of 1957, these high-fat, low-fiber rations were compared with the same high-fiber control ration used in previous studies, when fiber was the only variable. Because the estimated net energy supplied in each ration was kept to 105% of the cow's requirements, 21% less feed containing a total of 37% less fiber, was fed in the high-energy ration as compared to the control. The differences in favor of the high-fat, low-fiber ration were: milk, +2.1 lb. per cow per day; body temperature, −0.7° F.; respi-

ration rate, −3.6, and pulse rate, −2.7 per minute. The corresponding figures when the low-fat, low-fiber ration was compared with the high-fiber control ration averaged: +1.6 lb., −0.3° F., −6.4, and −4.0, respectively. The comparatively small differences in average daily milk production in favor of the high-energy ration for the 84-day summer trials were much less than the differences observed during the hottest weather.

P7. Effect of feeding tetraalkylammonium stearate to milking cows. A. C. LINNERUD, J. D. DONKER, AND J. B. WILLIAMS, University of Minnesota, St. Paul.

Ten cows, past peak milk flow and not more than 59 days pregnant, were paired and randomly allotted to control and experimental groups. A double-reversal feeding trial (28-day observation and 7-day change-over periods) was used. Chopped alfalfa-brome hay to satisfy appetite, and concentrate to balance nutrient intake, according to Morrison's Standard, were fed. Concentrate feeding was adjusted for each animal each period, to provide nutrients slightly above requirements. Tetraalkylammonium stearate (TAS) in a bone meal carrier (Dynafac) was included in the ration at 300 mg. TAS/cow/day. Daily milk weights and feed intakes were recorded. Weight changes were based on three-day weights at start and finish of each observation period. A two-day composite milk sample was analyzed each week for fat. Daily hay and bi-weekly concentrate samples were composited by periods for analysis by A.O.A.C. methods. Control values are followed by experimental values on a per-cow-per-day basis: milk production (4.0 fat corrected), 18.1 vs. 17.8 lb., concentrates consumed—6.8 vs. 7.0 lb., hay 23.0 vs. 23.2 lb., weight gained, 0.41 vs. 0.38 lb.

P8. Effect of certain measured environmental influences on the butterfat yield of Holstein-Friesian cattle.* E. E. STARKEY,** E. L. CORLEY, AND E. E. HEIZER, University of Wisconsin, Madison.

Environmental and butterfat production records were studied, using herd data collected for 3 yr. from 43 Holstein-Friesian herds in southern Wisconsin. Data were obtained monthly from DHIA field supervisors and through quarterly farm visits. Variables studied were: *(1)* butterfat yield (2×, 305-day, M.E.), *(2)* lb. of TDN fed daily, *(3)* lb. of protein fed daily, *(4)* nutritive ratio, *(5)* length of preceding dry period, *(6)* condition at freshening, *(7)* days carried calf, *(8)* calving interval, *(9)* herd size, *(10)* summer feeding, *(11)* housing, *(12)* milking practice, and *(13)* feeding practice. Variables 1–8 were recorded on each cow, whereas variables 9–13 were on a herd basis. The influence of Variables 2–13 on butterfat yield was estimated by multiple correlation techniques with 1,168 production and environmental records. Over-all herds, net partial regression coefficients of butterfat yield on Variables 2, 5, 7, 8, 9, 11, 12, and 13 were highly significant. Variables 2 and 13 were highly significant on a within-herd basis. Net partial regressions of butterfat yield on Variables 2 and 13 were 13.50 and 0.63 over all herds, and 23.31 and 0.49 within herds. Variation in butterfat yield associated with Variable 2 was 15% within herds, and 10% over all herds.

* USDA support.

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P9. Effect of feeding thyroprotein to dairy heifers. V. SINGH AND J. D. DONKER, University of Minnesota, St. Paul.

Five sets of identical-twin Holstein heifers, about 15 mo. old, were used. One member of each set received the control hay: grain ration, in which an alfalfa-grass hay was fed free choice and the grain was limited to 2 lb. daily and the other member of each set received thyroprotein in its grain. During a preliminary period, it was ascertained that from 7 to 7.4 g. per animal per day was required to increase pulse rate approximately 10% above normal, a level arbitrarily selected for comparison. The following data were gathered during a 6 wk. comparison period: heart rate (beats per minute), six-day average weights, weight gained—absolute gain (lb. per day) and relative gain (% increase per day), feed consumed—absolute quantities and lb. per cwt. The average values for the control data are followed by average experimental values: heart rate—89.3 vs. 96.6; average weight—666 vs. 651 lb.; weight gained—1.37 vs. 1.35 lb. per day and 0.22% vs. 0.22% increase per day; feed consumed—2 vs. 2 lb. grain daily and 16.8 vs. 16.4 lb. hay daily, and total feed per cwt. per day 2.52 vs. 2.52 lb.

P10. Methods of adjusting roughage intake data of dairy cows for differences in body size. R. E. MATHER AND A. A. RIMM, New Jersey Agricultural Experiment Station, Sussex.

Comparing roughage intake of cows of different sizes may lead to different conclusions, depending on the method of adjustment. Use of the ratio of intake to body weight (W) may overcorrect the data. Legates *et al.* proposed the use of regression for adjustment. Data were used from a study in which excellent alfalfa hay was fed ad libitum to cows of four breeds in 38 lactations, with limited corn silage and no grain, or grain at 1 lb. per 6 lb. FCM, or grain at 1:3. Regression adjustment provided a better fit than ratio adjustment, particularly for cows on the no-grain

ration, reducing the sum of squares of the unadjusted values by 66% compared to 24%. Limitations of data sometimes may prevent the adjustment by regression. Adjustment by ratio of intake to $W^{.73}$ gave adjusted values close to the regression-adjusted values on all three rations. These two methods ranked cows within ration the same in 26 of 38 cases. The reductions in sums of squares on the three rations were, respectively, 60, 48, and 71%, compared with 66, 56, and 71% by regression.

P11. Comparison of feeding corn cobs and a liquid urea-molasses-mineral mix with and without ethyl alcohol to yearling dairy heifers.* J. P. Bates, Jr., D. R. Jacobson, J. W. Rust, and D. M. Seath, University of Kentucky, Lexington.

Twenty dairy heifers, ten Holsteins and ten Jerseys, were divided into two equal groups and were individually fed ad libitum but separately a ration of ground corn cobs and a liquid urea-molasses mix with and without ethyl alcohol for a 168-day trial. Group M received the mix with ethyl alcohol, and group A the mix without ethyl alcohol. The average daily gains for 140 days, following a 28-day preliminary period, were 0.73 and 0.80 lb. for Groups M and A, respectively. This difference was not statistically significant. Groups M and A consumed 14.3 and 15.0 lb. of corn cobs and 5.5 and 4.8 lb. of liquid mix per animal per day, respectively. In the same order, pounds of feed per pound of grain were 27.1 and 24.8. Similarly, water consumption per 1,000 lb. body weight per day was 61.3 and 61.1 lb. when offered water continuously, and 56.7 and 54.4 lb. when watered once a day. For Groups M and A the average blood urea values were 17.27 and 14.59 mg. % and the plasma protein values were 5.86 and 5.78 g. %. Rumen fatty acid percentages were acetic, 68 and 67, propionic, 17 and 18, butyric, 10 and 10, valeric, 4 and 3, and the higher acid fraction, 0.9 and 0.9.

* This work was supported in part by Feed Service Corp., Crete, Nebraska.

P12. A proposed method for the measurement of the respiratory exchange and energy expenditure of grazing animals. W. P. Flatt, D. R. Waldo, and J. F. Sykes, Nutrition and Physiology Section, USDA, Beltsville, Maryland.

Large-animal energy metabolism studies have required the use of direct calorimeters, respiration chambers, closed-circuit spirometers, or long-term feeding trials in combination with body composition data. These methods are unsuitable for determining the energy expenditures of animals on pasture. A procedure has been devised to determine the O_2 consumption, CO_2 production, and energy expenditure of large animals for extended periods of time without restricting or confining them. The method involves the use of a light-weight (3.6 Kg) portable dry gas meter (Müeller-Franz Gas Meter, 100 L/min capacity, 1.5-in. intake outlet) with an aliquoting device, a 100 L. Douglas bag for collecting a continuous sample of expired air, harness, respiratory valve, and an animal fitted with a tracheal cannula. The total volume of expired air is measured by the meter, which is connected to the tracheal cannula and 0.1 or 0.2% of the expired air is continuously removed and collected in the Douglas bag for subsequent analysis for CO_2 and O_2. Preliminary studies have indicated that this method may be used for determining the energy expenditure for periods of approximately 12 hr. Longer experimental periods are made possible easily by changing the Douglas bags.

P13. Digestibility of orchard grass hays as influenced by two methods of nitrogen application.* R. A. Markley, J. L. Cason, and B. R. Baumgardt, New Jersey Agricultural Experiment Station, New Brunswick.

Four 7-mo.-old dairy heifers were used in a total collection digestion trial, ten-day preliminary and seven-day collection periods, employing a Latin-square design balanced for residual effects, with an extra period added to more effectively estimate carry-over effects. The four rations, fed twice daily, were second-cutting alfalfa and three first-cutting orchard grass hays; *(1)* low—0 lb. N/A, *(2)* low plus urea to equal protein content of the high, and *(3)* high—200 lb. N/A. A nitrogen balance was determined for each ration. The crude protein and digestion coefficients of protein for alfalfa were 16.45 and 71.41%; orchard grass hays—low, 6.47% and 44.99%; low plus urea, 15.82% protein equivalent and 77.77%; high, 15.07 and 70.72%, respectively. Ether extract and gross energy followed a trend similar to protein; whereas, the per cent of fiber and NFE decreased with increasing nitrogen fertilization. The digestion coefficients of protein, NFE, ether extract, and energy increased, whereas that of crude fiber decreased slightly with increasing nitrogen fertilization. The TDN and digestible energy (cal/g) of alfalfa were 49.04% and 2414; low, 47.46% and 2159; low plus urea, 55.44% and 2158, and high, hay 51.59% and 2511, respectively. The statistical analysis will be presented.

* Supported in part by a grant from E. I. duPont de Nemours and Co.

P14. Effect of high ash materials on use of the fecal chromogens method of determining digestibility. C. R. Richards and W. G. Crothers, University of Delaware, Newark.

The ash content of lima bean vine silage is very variable, owing to the method of harvest. With high-ash lots of silage, poor agreement between the chromogens method and conven-

tional or chromium sesquioxide methods of determining digestible dry matter has been noted. With 10.6% ash, digestible dry matter was 53.3 and 53.3%; at 19.2% ash, 53.3 and 48.5%; at 30.9% ash, 61.6 and 36.1% for the fecal chromogens and other methods, respectively. When the fecal chromogens were calculated to an organic matter base and digestion coefficients determined for the organic matter, the agreement between methods was much more satisfactory: 54.8 and 57.4; 57.2 and 57.8, and 53.5 and 54.7, respectively.

P15. Trace-mineral supplementation for dairy cows. E. G. Moody, J. M. Conover, and A. P. Cook, Arizona State College, Tempe.

Using ten pairs of Holstein-Friesian cows in a 22-wk. reversal trial, no effect could be attributed to trace-mineral supplementation of a ration of high-quality alfalfa hay, simple grain mix, bone meal, and salt from measurements of milk production, body weight changes, general condition, hemoglobin, hematocrit, and in the serum: sodium, potassium, calcium, phosphorus, magnesium, chlorine, carotene, and vitamin A. All values, with a few individual exception, were within, or above normal published values. Cows preferred fortified to unfortified salt about 2:1, when given free access to both.

P16. Magnesium sulfate, grain, or alfalfa hay as sources of magnesium to calves fed whole milk. J. W. Thomas and M. Okamoto, Nutrition and Physiology Section, USDA, Beltsville, Maryland.

Whole milk at the rate of 10 to 12% of body weight was fed to 12 calves. When serum magnesium concentration became subnormal (48–131 days of age), magnesium in amounts equal to that supplied by the milk was added as magnesium sulfate, grain, or alfalfa hay. Serum magnesium concentrations gradually decreased as the experiment progressed. The extent of this decrease from the fourth to the tenth month was 1.16, 0.48, and 0.45 mg. magnesium per 100 ml. serum for the $MgSO_4$, grain- and hay-supplemented calves, respectively. Magnesium concentration in ash of rib-biopsy sections taken at 240 days of age was 0.66, 0.67, and 0.75%, respectively, for the $MgSO_4$, grain- and hay-supplemented calves. Magnesium in the ash of rib ends at death (280–467 days of age) was 0.40, 0.54, and 0.69%, respectively. A dietary intake of magnesium of 0.8 to 0.9 gm/cwt was inadequate when half the magnesium was supplied by whole milk and half by either $MgSO_4$, grain, or hay. In this trial, the magnesium in grain and hay was apparently utilized better than magnesium in $MgSO_4$, but the differences were not statistically significant.

P17. Determination of methoxyl in the presence of carbohydrates. W. C. Jacobson and H. G. Wiseman, Nutrition and Physiology Section, USDA, Beltsville, Maryland.

A modification of the apparatus designed by Clark has been used for the determination of methoxyl in the presence of carbohydrates. Modifications include a glass bubbler for sweeping the CO_2 through the hydriodic acid, and a coarse-fritted glass bubbling device for passing the gas through the acetic acid-bromine-potassium acetate solution in the receiver. The trap contained a 1% starch solution to hold any free iodine. Distillations were carried out at steam-bath temperatures. It has been demonstrated that both Bureau Standards dextrose and α-cellulose give a methoxyl value at a linear rate for at least 8 hr. Corrections are made for the presence of carbohydrates by making an additional collection of equal duration and subtracting this value from the first run. Ninety minutes are required to give quantitative recovery of vanillin in the presence of 0.5 g. cellulose, when the value of the second 90-min. collection is subtracted as a blank. When lignin in hay is estimated from methoxyl values, the error due to carbohydrates must be considered.

P18. Determination of sugars in silage with micro-Somogyi copper reagent. H. G. Wiseman, J. C. Mallack, and W. C. Jacobson, Nutrition and Physiology Section, USDA, Beltsville, Maryland.

An improved procedure has been devised for the determination of sugars in silage. The extractions are made in a Waring Blendor, with 80% ethyl alcohol. The extracts are filtered through a bed of Darco G-60 and Celite 535, to give practically colorless solutions. Aliquots are stirred with a mixture of ion-exchange resins, Duolite A-4, and Dowex-50. The deionized aliquots are dealcoholated on the steam bath, made to 1- normal with hydrochloric acid, and allowed to stand overnight to invert. The inverted solutions are neutralized, made to volume, and 10-ml. aliquots heated with 10 ml. of Somogyi's copper reagent (1945). After cooling, the contents are titrated with thiosulfate, stirring being conveniently accomplished with a magnetic stirring bar. Since the sugar solutions have been clarified before bringing nearly to dryness to dealcoholate, economy of volume size is possible; troublesome solution and filtration of gum residues are avoided, and duplication improved.

P19. Effect of adding water to the concentrate mix on maximum milking rate, average milking rate, and eating rate. E. W. Hupp and R. C. Lewis, Michigan State University, East Lansing.

The maximum rate of milk flow, average milking rate, and concentrate eating rate were determined for 16 Brown Swiss cows in loose-housing for six consecutive milkings. Following these observations, water was added to the concentrate mix at the rate of 1.5 lb. per pound of

feed. After a three-day adjustment period, the effect on the above characteristics was determined for six additional milkings. The average maximum rate of milk flow was 3.73 lb. per minute on dry feed and 3.60 lb. after water had been added. The average rate of milking was 2.19 lb. per minute on dry feed and 1.92 lb. per minute on wet feed. Neither of these differences was significant. Eating rate increased significantly ($P = >0.01$) when water was added to the concentrate mix. On dry mix the cows ate 0.63 lb. per minute, whereas the rate increased to 0.90 lb. with the addition of water. Five days later the rates were determined for two milkings. The maximum milking rate was 3.83 lb., the average rate 2.0 lb., and the average eating rate, 0.56 lb. per minute. These results support reports that the concentrate eating rate can be increased by the addition of water. No evidence of an effect on milk flow was found.

P20. Effect of some weather factors on daily milk and fat production. F. N. Dickinson, USDA, and H. W. Norton and R. W. Touchberry, University of Illinois, Urbana.

One hundred and one dairy cows from a single herd, including purebred Holsteins and Guernseys and Holstein-Guernsey crossbreds, were sampled for A.M. and P.M. milk yield and composite butterfat test, at ten-day intervals from May 1 to September 30, for 5 yr. The amount of fat produced each sampling day was calculated. The maximum, minimum, and mean temperature, 1 P.M. relative humidity, and mean wind-speed for each sampling day were taken from local weather bureau records. An individual quadratic curve was fitted separately to each type of data for each cow. The deviations from each estimated curve were calculated and summed over cows within dates for the same summer. These within-date sums of deviations from fitted quadratics were analyzed by the method of multiple regression, to ascertain the effect of maximum, minimum, and mean temperature, relative humidity, and mean wind-speed on milk and fat production.

P21. A study of milk flavors. C. F. Foreman, E. W. Bird, F. E. Nelson, W. S. Rosenberger, and M. R. Lambert, Iowa State College, Ames.

The milk from each of 169 cows from five breeds in the College herd was tasted monthly during 1957, to establish flavor characteristics and patterns. Three experienced judges independently gave numerical flavor scores (40 perfect) and listed the type of flavor for each sample. Marked differences in flavor and flavor scores existed among animals under the same managemental conditions. The mean score for all cows varied from 34.7 in January to 35.9 in June, September, November, and December. Feed was the most common flavor noted and since normal feed flavor scores varied over a fairly wide range, it suggests a need for study and cataloging of undesirable and acceptable feed flavors and flavor intensities. Common flavor defects other than feed were cowy, oxidized, flat, unclean, and salty. Cowy flavors occurred more often during the winter. Oxidized type flavors were not confined to winter, although the defect was more pronounced during this period. These flavors occurred most frequently during early lactation, although some cows showed them throughout a lactation. The milk from some cows characteristically contained certain flavor defects. The incidence of rancidity was quite low. Further data on flavor patterns will be presented.

P22. Effect of thyroprotein on metabolic and productive function of lactating Jersey cattle exposed to hot and cool weather. J. E. Johnston, G. A. Hindery, G. T. Schrader, and F. B. Hamblin, Louisiana State University, Baton Rouge.

Twenty lactating Jersey cows were divided into four groups on the basis of milk production. Two groups were placed in the climatic control chamber and two groups in an open shed. Conditions in the chamber were adjusted to simulate subtropical summer weather. Conditions in the open shed generally ranged between 40 and 65° F. One group of animals in each location was fed 1½ g. of thyroprotein per 100 lb. body weight. All animals were fed 3 lb. of grain in addition to 1 lb. per 4 lb. of FCM and roughage ad libitum. Thyroprotein feeding resulted in increases in heat production, body temperature, respiratory activity, milk production, and fat percentage. During 40 days under hot conditions, control animaals lost an average of 55 lb. each, whereas thyroprotein animals lost an average of 136 lb. Under cool conditions, controls gained 5 lb. each, whereas thyroprotein animals lost 25 lb. Under hot conditions, controls declined in milk production initially, whereas thyroprotein groups maintained production for the first 20 days then declined, showing severe distress associated with high body temperatures, decline in roughage consumption, and loss of weight.

P23. Effects of high levels of vitamin D_2 fed prepartum on the incidence of parturient paresis in dairy cattle. J. C. Dell and B. R. Poulton, University of Maine, Orono.

Studies were begun in 1956 to determine the value of high-potency irradiated ergosterol, about seven million U.S.P. units per gram, as a preventive for parturient paresis in dairy cattle. Thirty-one herds of Jersey cows are being used. All cows calving for the third time or more are assigned to four experimental groups. Groups I and IV include only animals with a previous history for parturient paresis. Groups II and III include only animals with no previous history for parturient paresis. Groups I and II serve as treatment groups, whereas

Groups III and IV are controls. The preventive feeding program used is as follows: Beginning approximately 72 hr. prepartum and continuing until 48 hr. postpartum, all animals in the treatment groups received 30 million U.S.P. units of vitamin D_2 daily in their grain. Thus far, data have been collected for over 400 parturitions. The percentage incidence of clinical cases of parturient paresis (requiring calcium therapy) has been as follows: Group I, 32.15%; Group II, 9.15%; Group III, 19.69%, and Group IV, 65.38%.

P24. A calcium lactate–aluminum hydroxide preparation as a milk fever preventive. G. M. Ward, Colorado State University, Fort Collins.

A commercial product* containing calcium lactate and aluminum hydroxide, with and without vitamin D_3 to supply about 500 units per day, was fed to dairy cows for an estimated eight days prior to parturition. This product supplied about 20 g. of calcium per day. Seventeen cows in the University herd with a previous history of milk fever received this material by capsule or drench, and blood samples were analyzed for calcium and phosphorus. Five of these cows developed milk fever and three suffered relapses. Serum calcium and phosphorus levels were no different than those which have been reported previously for cows under these conditions. All three cows which received the material for longer than 11 days developed milk fever. Thirty-two cows in private herds with a previous history of milk fever were fed the product in their grain. Three of these cows were treated for milk fever. Additional vitamin D had no advantage over the product without this vitamin.

* The product Paracalein and financial support was furnished by Warner-Chilcott Laboratories, Morris Plains, New Jersey.

P25. Production of polyvalent antibodies in milk absorption and prophylactic and therapeutic value in calves. Mohammad Sarwar, W. E. Petersen, and Berry Campbell, University of Minnesota, St. Paul.

Polyvalent-killed antigen were prepared from the cultures of *Pasteurella, Escherichia coli, Streptococci, Corynebacterium, Salmonella enteritidis, Staphylococcus albus* and *aureus,* and *Aerobacter aerogenes,* and were infused intramammarily in 18 cows with antibody production against each organism. The polyvalent antibody milk was fed to eight calves from 2 to 5 mo. of age and, in two to three days of feeding, specific antibodies were demonstrated in the blood. In test for the prophylactic value of the antibody so produced, the polyvalent antibody milk was given to five experimental calves for seven days before beieng challenged with 60 billion cells of pathogenic *E. coli* per ovum; whereas, the control calves continued on the normal milk. The five experimental calves survived and the controls died. Test for the therapeutic value of gamma globulin prepared from polyvalent antibody milk when it was intravenously injected (0.5 grain per 100 lb. of body weight) for three days at the height of the disease, resulted in prompt recovery of all five calves. Two controls died and two survived with prolonged illness and acute arthritis.

P26. Antitryptic factor and antibody absorption. W. E. Petersen, Mohammad Sarwar, and Berry Campbell, University of Minnesota, St. Paul.

Experiments based on the antitryptic factor in soybean flour were set up to test the validity of the concept of antibody absorption from the G.I. tract in adult animals. Antibody absorption occurred because the proteolytic enzymes are overwhelmed, permitting unaltered, immune protein to enter the small gut. Twenty-eight adult rabbits were used, in which varying levels of toasted soybean flour (antitryptic factor destroyed) for the controls and raw soybean flour for the experimentals were used, admixed with the polyvalent antibody milk fed ad libitum. Levels of the soybean flour varied from 1 to 20% by weight of the milk. The polyvalent antibody milk was prepared from antigen consisting of equal numbers of *Escherichia coli, Salmonella enteritidis, Streptococcus hemolyticus, Staphylococcus albus, Staphylococcus aureus, Corynebacterium,* and *Pasteurella bovisepticus.* Only antibodies against *E. coli* and *S. enteritidis* were titered. The milk agglutinated at 1 to 1,000 dilution. All rabbit sera were negative before the experiment began. All animals on antibody milk acquired antibodies in their sera. Those on milk with the antitryptic factor picked up sera antibodies at five- to more than tenfold the levels in those on the heated soybean flour, where the antitryptic factor was destroyed.

P27. Milk residues associated with use of experimental animal systemic insecticides. J. E. Casida, R. P. Niedermeier, J. B. Knaak, W. C. Dauterman, H. R. Krueger, and F. W. Plapp, University of Wisconsin, Madison.

Lactating cows were treated with three experimental animal systemic insecticides containing radioactive phosphorus. One to three cows were used per compound. The rate of secretion of the insecticides and/or their metabolites in the milk was studied. Materials studied were: Trolene or ET-57 [0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate] · Dimethoate or CL 12,880 (0,0-dimethyl S-methylcarbamoylmethyl phosphorodithioate) and Co-Ral or Bayer 21/199 [0,0-diethyl 0-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate]. The first two materials were given orally and the third applied dermally. In all cases, the peak secretion of insecticide in the milk occurred within 12 hr. after treatment. The highest milk residues were encountered with Trolene and the lowest

with Co-Ral. These experimental insecticides should not be tested on dairy cattle when the milk is to be marketed. This study was carried on in conjunction with metabolism and tissue residue studies on thees organo-phosphorothioates.

P28. Comparison of the Orange G dye and Kjeldahl methods for determining milk protein. J. M. TREECE, L. O. GILMORE, AND N. S. FECHHEIMER, Ohio Agricultural Experiment Station, Wooster, and The Ohio State University, Columbus.

A slight modification of the Orange G dye protein method of Udy was compared to the standard macro-Kjeldahl procedure, using 68-wk.-old composited samples of whole milk from Holstein, Jersey, and Guernsey cows of different ages and in different stages of lactation. From the unbound dye, which is measured colorimetrically, an estimate of the protein content can be made either graphically of by use of a predetermined constant. By using constants that disregard NPN, the sample means (3.16 and 3.16%) and standard deviations (0.62 and 0.60), respectively, for the dye and Kjeldahl methods, are not significantly different ($P < .001$). This shows that the dye method is as accurate as the Kjeldahl method and requires less than one-fourth the time. Furthermore, it is much less expensive than the Kjeldahl method, is specific for protein, and is easily adaptable to field use. This method is being further investigated, for use in estimating the casein fraction of milk.

P29. Milk preservative for solids-not-fat determinations.* M. L. NELSON, M. N. DEUTSCH, AND C. M. CLIFTON, University of Minnesota, St. Paul.

A survey was made of materials commonly used as preservatives for milk samples, in search of a suitable material for use in samples to be tested for solids-not-fat. Determinations were made by use of the small Watson lactometer, using 4-oz. samples, preheated and read after holding 5 min. in a thermostatically controlled water bath at 102° F. ±0.5°. Each preservative tested was evaluated in terms of fresh, unpreserved samples, by means of a split-sample technique. The qualities desired were a material which would not cause a significant change in specific gravity, and would preserve samples for at least seven days. Disodium phosphate, bichloride of mercury, sodium dichromate, and potassium dichromate produced from 0.26 to 0.90 increases in per cent S.N.F. over duplicate control samples. Zepherin failed to preserve samples seven days. Formalin used in the proportion of one part of formaldehyde to 1,500 parts of milk, produced slightly higher lactometer readings, averaging 0.03% S.N.F. ($P > 0.05$). This quantity of formaldehyde-preserved samples was taken under field conditions without refrigeration.

* This work was done in cooperation with the Dairy Husbandry Research Branch, USDA.

P30. In vitro cultivation of bovine mammary gland in tissue cultures. K. E. EBNER AND B. L. LARSON, University of Illinois, Urbana.

This paper is a preliminary report on investigations under way in this laboratory to study the biosynthesis of milk proteins in the secretary cells of the bovine mammary gland. As a part of this investigation, cell explants obtained by a collagenase dispersion of bovine mammary tissue were placed and maintained in culture using defined nutrients plus blood serum and/or plasma for the cultivation media. As far as the authors are aware, this is the first report of the in vitro proliferation of cells derived from the bovine mammary gland in tissue cultures. The various aspects of its cultivation will be discussed.

P31. Progress report on experimental stimulation of milk secretion.* C. W. TURNER AND H. L. RUPPERT, JR., University of Missouri, Columbia.

Study of the experimental induction of growth of the cow's udder and the initiation of milk secretion in normal and infertile heifers has been continued. Estrogens (100 μg. estradiol benzoate by injection or 10 mg. diethylstilbestrol orally) and 100 mg. progesterone have been administered daily for 180 days. Then, progesterone has been discontinued and 3 mg. estradiol benzoate injected for 14 days to initiate lactation. Some infertile heifers have conceived during the ensuing lactation period. Comparison of experimental maximum milk yield and normal milk production in these animals will be presented. A report on success of inducing experimental lactation after normal lactation will be presented. Why fremartin heifers generally fail to respond to experimental treatment will be discussed.

* These investigations were supported in part by grants of the U. S. Atomic Energy Commission [Contract No. AT(11-1)-301].

P32. Optimal hormone levels and lactation. C. E. GROSVENOR* AND C. W. TURNER, University of Missouri, Columbia.

A study was conducted to obtain uniform maximum milk yields per timed nursing in populations of lactating rats by the administration of optimal amounts of growth hormone (GH) and oxytocin, alone and in combination. Each rat on the 14th day of lactation was isolated from its litter for 10 hr., then replaced and allowed to nurse 30 min. Weight of milk thus obtained, expressed as per cent litter body weight, averaged 4.3% with normal distribution

for control animals. GH injected daily from the 7th to 14th days of lactation resulted in significantly greater yields (6.0%). Oxytocin injected at time of nursing into untreated rats similarly increased milk yields significantly (5.8%). Distribution curve of results following treatment with oxytocin or GH was shifted to the right and the range of values more constricted. There was further reduction in variance of milk yields in GH-treated animals injected at the time of nursing with oxytocin. Rather uniformly high milk yields were obtained. The results of this study indicate that individual variation among lactating animals may be greatly reduced by administration of optimal hormone levels, for it is probable that milk secretion and milk let-down is thereby enhanced in poor-lactating animals.

* Postdoctoral Fellow of N.I.H. This investigation was supported in part by a research grant from the Public Health Service.

P33. Protein-bound iodine and I^{131} extrapolation technique as methods of determining thyroid activity in dairy cows. F. B. Hamblin, J. E. Johnston, and G. Schrader, Louisiana State University, Baton Rouge.

Eight mature nonlactating cows (four Jerseys and four Holsteins) were placed in a climatic control chamber in a thermally neutral environment, in October, 1957. Serum protein-bound iodine was determined at five-day intervals, prior to I^{131} injection, by the Hycel method. Cows were injected subcutaneously with approximately 100 μc. of I^{131} per 100 lb. body weight. Maximum uptake of I^{131} by the thyroid occurred within 72 hr. Five days after initial injection, thiouracil was administered orally at the rate of 1 gm/cwt to partially prevent reutilization of I^{131}. Daily subcutaneous injections of l-thyroxine were begun after a release curve had been established. Thyroid counts were taken daily and corrected for decay and instrument adjustment. Dosages were increased (except for one cow which had to be decreased because of lower secretion rate) on alternate days until there was no further release of I^{131} from the thyroid. By this technique the thyroid secretion rate of individual cows was found to vary from 0.077–0.195 mg/cwt. The amount of PBI varied from 5.76–6.57 μg.%. Variability in thyroid secretion rate as measured by I^{131} was much greater than by PBI procedures. The two methods were found to have a positive correlation of 0.59.

P34. Turnover of I^{131}-labeled thyroxine in cattle.* B. N. Premachandra, G. W. Pipes, and C. W. Turner, University of Missouri, Columbia.

The determination of thyroxine secretion rate in cattle by replacement therapy involves repeated injections of thyroxine in increasing dosages until the release of I^{131} from the thyroid is completely inhibited. It was essential, therefore, to study the carry-over of unmetabolized hormone from one injection to another. I^{131}-labeled thyroxine was injected intravenously into eight dairy cows and calves, and daily radioactive measurements of the plasma and urine were made with a well counter. Thiouracil was administered to prevent reutilization of I^{131} by the thyroid of metabolized thyroxine I^{131}. Otherwise, reutilization would mask the true measurements of the biologic half-life of thyroxine in blood and consequently the appearance of I^{131} in the urine. The $T\frac{1}{2}$ of labelled thyroxine ranged from 2.0 to 2.75 days in plasma and the $T\frac{1}{2}$ for disappearance of I^{131} in urine ranged from six to eight days. From the rapidity of the disappearance of thyroxine from the blood, it is inferred that the estimations of thyroxine secretion rate in cattle are not exaggerated. This also indicates that thyroxine from thyroprotein is metabolized rapidly.

* These investigations were supported in part by grants of the U. S. Atomic Energy Commission [Contract No. AT(11-1)-301].

P35. Efficiency of absorption of thyroxine in thyroprotein from the gastrointestinal tract of lactating dairy cattle. J. P. Mixner and H. D. Lennon, Jr., New Jersey Agricultural Experiment Station, Sussex.

Seven lactating Holstein cows were used to determine the efficiency with which thyroxine in thyroprotein was absorbed from the gastrointestinal tract. The criterion of absorption efficiency was the elevation in the levels of plasma protein-bound iodine (PBI) by the orally administered thyroprotein, as compared to intravenously injected l-thyroxine. At different times each animal received intravenous l-thyroxine (25.2 mg.) and oral thyroprotein (25 g. containing 265 mg. of thyroxine), in order to have within-animal comparisons. At least two blood samples were collected from each animal during the disposal phase of peripheral thyroxine metabolism. The theoretical level of PBI at zero-time, with reference to injection of thyroxine and the oral administration of thyroprotein, was calculated. The PBI increase per milligram of thyroxine administered was then determined for the thyroxine from the two sources. With the injected thyroxine being rated as 100% efficient, the relative efficiency of absorption of the thyroxine in thyroprotein was evaluated. The mean efficiency of absorption of the thyroxine from the orally administered thyroprotein was $12.3 \pm 1.33\%$, with a range of from 8.30 to 17.4%, standard deviation being 3.53% and the coefficient of variation, 28.7%.

P36. Magnitude and variations in plasma PBI values in Ayrshire cattle at the West Virginia Agricultural Experiment Station. R. O. Asplund, G. A. McLaren, and I. D.

PORTERFIELD, West Virginia University, Morgantown.

Samples of blood ranging in number from three to eight were collected from two to five days apart, from each of 45 Ayrshires at 3-mo. intervals, from ages 3 to 18 mo. All samples were analyzed for plasma protein-bound iodine (PBI) by the method of Brown *et al.* PBI values were also determined on the dams of these animals. Unusually high PBI values were encountered in most individuals. Levels from 15 to 30 μg. per 100 ml. of plasma were common, and values as high as 75 μg. were obtained. These high levels were seasonal, occurring mostly in the spring, with a few in the late fall. This was found in all age groups, but none showed symptoms of hyperthyroidism. These results have been confirmed by other laboratories, using both the same and different analytical methods. The between-day variation of individuals was too large to detect significant differences between individuals. Some evidence indicates that grass silage may affect PBI levels in a positive manner.

P37. An isotope dilution technique for the determination of adrenocorticotropic hormone secretion rate in dairy cattle. W. F. WILLIAMS, University of Maryland, College Park.

Determination of the secretion rates of the various hormones is essential to the understanding of the endocrine physiology of dairy cattle and other animals. The development of methods for making these determinations is of importance to further advancements in this field. The usfulness of isotopic labeling of a protein pituitary hormone, ACTH, for the measurement of its secretion rate has been studied. An isotope dilution technique, involving a constant intravenous infusion of labeled ACTH and the determination of ACTH specific activity in periodic blood samples, has been developed for the secretion-rate determination. The possibility of the use of this technique for the determination of other hormonal secretion rates will be discussed.

P38. Some physiological responses of the bovine to dihydrotachysterol and parathyroid extract administration. K. A. KENDALL AND K. E. HARSHBARGER, University of Illinois, Urbana.

In a preliminary study with nonlactating Jersey and Guernsey cows, dihydrotachysterol was administered intramuscularly at levels of 6 and 9 mg. per animal while parathyroid extract at the rate of 1,000 units was administered. During a 24-hr. period, following administration, blood samples were taken, and fecal and urinary excretion rates were measured at regular intervals. Dihydrotachysterol appeared to have no diuretic effect, whereas urinary volume was doubled following parathyroid extract administration. Blood serum calcium and phosphorus showed little or no change when parathyroid was administered, whereas calcium appeared to increase slightly and phosphorus declined when dihydrotachysterol was injected. Both urinary phosphorus and calcium excretion rates were increased when parathyroid extract was injected; whereas, urinary phosphorus excretion in relation to calcium appeared to decline after the first 12 hr. following injections of dihydrotachysterol. Fecal calcium to phosphorus ratios in the dihydrotachysterol-treated cows appeared to increase from 1:3.5 to 1:6.3 at 12 hr. after injection. Fecal calcium and phosphorus values as related to parathyroid extract administration are being determined. It would seem from these preliminary observations that dehydrotachysterol and parathyroid extract, at the levels administered, did not produce similar physiological responses in the bovine.

P39. Release of ammonia nitrogen from uric acid, urea, and certain amino acids in the presence of rumen microorganisms. C. G. LOOPER AND O. T. STALLCUP, Arkansas Agricultural Experiment Station, Fayetteville.

The amino acids, arginine and glycine, and urea were each placed in artificial rumens in amounts so as to have equal nitrogen content. The ammonia in the flask containing glycine was not significantly different from a control flask containinly only inoculum. The release of ammonia from arginine was intermediate between that released from urea and the control. Morea (a urea-molasses-alcohol mixture) chicken litter (containing a high percentage of uric acid) and glycine were each fed to a fistulated steer, in conjunction with cottonseed hulls for roughage, in amounts so as to have equal nitrogen intake. The ammonia present in the rumen liquor, expressed as mgm/100 ml., at selected intervals after feeding for morea, chicken litter, and glycine, respectively, was as follows:

2 hours—	11.95,	13.40,	and 1.30
4 hours—	10.85,	8.1,	and 1.30
6 hours—	5.65,	3.40,	and 1.50
9 hours—	3.45,	1.30,	and 1.60
12 hours—	2.18,	1.00,	and 0.80

The ammonia released from glycine was approximately that released on a control diet when only cottonseed hulls were fed.

P40. The B-vitamin and N requirements of Bacterioides succinogenes, an important ruminal cellulolytic bacterium. HILDA CHU AND M. P. BRYANT, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Previous studies on this organism showed that a fermentable carbohydrate, CO_2, and a two-component system of certain branched- and straight-chain volatile fatty acids were required for growth. The growth medium also contained minerals, purines, and pyrimidines, vitamin-free enzymatic hydrolysate of casein, and cys-

teine. Deletion of purines and pyrimidines had no effect on growth of five strains tested. Three strains gave optimum growth with only biotin of the B-vitamins present; the other two strains, with biotin and PABA. Biotin was a strict requirement of four strains and highly stimulatory to the other. PABA was highly stimulatory, but not a strict requirement of two strains. Casein hydrolysate could be replaced by a mixture of 19 amino acids or by cysteine-HCl alone. In the latter case, growth was good but sometimes delayed. NH_4^+ was required for growth, even when purines and pyrimidines and 19 amino acids were present in the medium. Glutathione replaced cysteine, but ascorbic acid and sodium sulfide were inhibitory. Work on N and S requirements are continuing. The results indicate that *Bacterioides succinogenes*, though a difficult organism to grow in pure culture, has a relatively highly developed ability to synthesize cellular constituents.

P41. Metabolism and absorption of butyrate-1-C^{14} by the perfused rumen. R. E. McCarthy, J. C. Shaw, Jeanne McCarthy, and S. Lakshmanan, University of Maryland, College Park.

Butyrate-1-C^{14} was added to the excised rumen of a 50-lb. goat (sacrificed 2 hr. after a normal feeding of hay and grain) at the beginning of an 80-min. perfusion study. Total production of rumen organic acids during perfusion in mg. was: acetic 641, propionic 306, butyric 268, valeric 107, formic 48, and lactic 306. Total increases (absorption) in perfusion blood organic acids substances in mg. were: acetic 363, propionic 117, butyric 107, valeric 25, and lactic 335; blood formic acid and glucose decreased 33 and 145 mg., respectively (ruminal tissue utilization), whereas no change occurred in blood acetone bodies. The final percentage distribution of radioactivity within the rumen showed some recycling of labeled butyrate. Of the total activity remaining, 62% was in butyrate, 25% in acetate, and 10% in propionate. Following perfusion, the activity in blood was 72% butyrate, 8.4% propionate, 7.9% acetate, and 9.7% lactate. There was little or no activity in blood formate, valerate, acetoacetate, β-hydroxybutyrate, carbon dioxide, and glucose (osazones). It appears that absorption of organic acids is a reflection of rumen production of these acids. Butyric acid is not converted to blood acetone bodies during absorption.

P42. Effect of KNO_2 and KNO_3 on in vitro cellulose digestion by bovine rumen microorganisms. V. H. Viebrock and C. P. Merilan, University of Missouri, Columbia.

Manometric studies were made on the in vitro utilization of a cellulose substrate (Solka-floc) by suspensions of rumen microorganisms from fistulated dairy heifers maintained on a roughage ration. The inclusion of low levels of KNO_3 in the reaction flasks stimulated gas production by 9.6, 5.8, and 3.3%, respectively, for concentrations of 0.125, 0.25, and 0.5% KNO_3 on a dry matter basis. However, the change in the portion of total gas production, which could be attributed to cellulose digestion, indicated 27.3, 32.4, and 27.8% increase in apparent cellulose digestion at these potassium nitrate concentrations. Higher levels of KNO_3, 1.0, 2.5, and 5.0%, resulted in decreased total gas production, as well as in 26.1, 48.9, and 80.0% decreases in apparent cellulose digestion. Potassium nitrite concentrations, ranging from 0.01 to 0.1% of dry matter, inhibited apparent cellulose digestion 7.9 to 67.0%, respectively, whereas 5.0% KNO_2 decreased the cellulolytic activity by 91%.

P43. Volatile fatty acid production and absorption in the perfused rumen. C. L. Davis, R. E. Brown, J. R. Staubus, and W. O. Nelson, University of Illinois, Urbana.

Three mature goats were starved 24 hr., then fed 2 lb. of hay and 1 lb. of concentrate. One hour after feeding the goats were anaesthetized and bled via carotid cannula until no further blood could be obtained. The rumens were removed and perfused first with saline rinse and then with the homologous heparinized blood. The average rate of VFA production was acetic, 2.61 g/hr; propionic, 1.16 g/hr; and butyric, 0.98 g/hr. The average absorption rates were acetic, 140 mg/hr; propionic, 72 mg/hr; butyric, 64 mg/hr. The low rates of absorption may be attributed to a slow rate of blood flow, which averaged 17 ml/min for the three perfusions. In contrast to reports in the literature, the relative proportions of VFA in rumen vein blood (A, 51, P, 26, B, 23) were nearly identical to those in the rumen fluid (A, 55; P, 22; B, 23). Glucose utilization by rumen tissue was demonstrated by A-V glucose differences of 6–10 mg.%. When venous blood was recirculated during one perfusion, there was a threefold increase in rate of blood flow, but there was no VFA absorption into the recirculated blood and no glucose uptake by the rumen tissue.

P44. Effects of various feeds on volatile fatty acid production in vitro. R. G. Hinders and G. M. Ward, Colorado State University, Fort Collins.

Rumen bacteria obtained from cows on rations of (I) brome grass hay, (II) brome grass hay and grain, and (III) alfalfa hay and grain were used for in vitro studies of volatile fatty acid (VFA) production. VFA content and pH were determined using excellent alfalfa, poor alfalfa, brome grass hay, mixed alfalfa and grass hay, mixed excellent alfalfa and concentrate, and concentrate alone as the substrates. Rumen bacteria from animals on Ration III with excellent alfalfa as the substrate

produced the highest concentration of acids with 140.2 μM/ml (50.3% acetic, 34.7% propionic, and 15% butyric and higher acids). The lowest value was produced by Ration I bacteria with poor alfalfa as the substrate (107 μM/ml; 52.7% acetic, 29.8% propionic, and 17.4% butyric). The acetic, propionic, and butyric acid percentage of VFA and the pH, respectively, for Ration I was 58, 24, 16, and 6.6; for Ration II—52.7, 29.9, 17.4, and 6.4, and for Ration III, 63.8, 20.2, 16.0, and 6.3.

P45. Effect of ethanol on rumen fermentation. T. R. Lewis, R. S. Emery, and J. P. Everett, Michigan State University, East Lansing.

Ethanol (800 ml., 47.5%) was administered via rumen fistula during the once-daily feeding of, (1) 14 lb. timothy hay plus 4 lb. of equal parts ground corn and corn cobs or, (2) ration 1 with 3% urea replacing corn or, (3) 20 lb. alfalfa hay, to twin 1,100-lb. cows. Each ration was fed with and without alcohol to each cow for 14-day periods. Four per cent of ground ration was added to coarsely strained rumen liquid and incubated for 3 hr. in vitro. Ethanol was not fermented in vitro but disappeared from the rumen with a half-time of 2 hr. A maximum rumen ethanol concentration of 500 mg. per cent was indicated. Blood contained 75 mg. per cent ethanol 2 to 4 hr. after administration. Ethanol depressed rumen dry matter content, and utilization of radioactive inorganic sulfate for cystine synthesis in vitro without affecting in vitro ammonia utilization or synthesis of methionine or glutathione. Total gas production in vitro was depressed by ethanol.

P46. Efficiency of rumen fermentation as affected by corn and adaptation to ration. R. S. Emery, C. K. Smith, T. R. Lewis, and June DeHate, Michigan State University, East Lansing.

A 900-lb. rumen fistulated steer was fed daily *(1)* 18 lb. alfalfa hay, or *(2)* Ration 1, with corn substituting for 10% of the alfalfa T.D.N., or *(3)* corn substituting for 20% of the alfalfa T.D.N. Inocula (200 ml. coarsely strained rumen liquid), taken from two to five and from 19 to 21 days after each ration change, was incubated in a flask with 10 g. finely ground Ration (1) or 9.4 g. Ration (2) or (3). Three milliliters sulfuric acid were added to separate flasks after 0, 3, and 6 hr., to stop the fermentation. Inoculum from Ration (1) was also fermented with each substrate. Fermentation products were converted to their caloric equivalents, assuming 0.0035 Cal. per milliliter CO_2 produced. Fatty acid production was increased by corn and by adaptation to the ration, largely owing to increased acetic acid production. Caloric rations of CO_2 and CH_4 were 2.3 and 1.6 for the first and second 3 hr. of fermentation for the eight trials. The efficiency ratios (calories of volatile fatty acids to calories of gas) were 18.7, 17.4, and 22.5 for Rations (1), (2), and (3). These were not affected by adaptation to the ration.

P47. Physical and chemical changes in bovine saliva and rumen liquid with different hay: grain ratios. C. K. Smith, R. S. Emery, R. M. Grimes, C. F. Huffman, and C. W. Duncan, Michigan State University, East Lansing.

Saliva was collected at the cardial orifice and rumen liquid samples were taken before feeding and 3 hr. after feeding from two cows on 20, 40, 60, 80, and 100% hay rations. The viscosity (mean 104 cp.) was greater with 20 and 80% hay rations than with 60 and 100% hay rations. The viscosity after feeding was about twice that obtained before feeding. The pH (mean 8.21) tended to increase the proportion of hay increased. Ash (mean 0.7337 g/100 ml.) tended to be higher at both extremes of the hay:grain ratio. Organic matter (mean 0.3854 g/100 ml.) tended to be low at both extremes of the hay: grain ratio. Total nitrogen was 20.05 mg.% for the 20% hay rations and averaged 14.88 mg.% for the 40–100% hay rations. Hexosamine (mean 4.25 mg.%) was higher on the high grain ration. The rumen liquid samples were centrifuged at 25,000 G and the supernatant analyzed. The dry matter mean was 1.71% and the ash content mean was 0.79%. Sodium (mean 234 mg.%) increased with increasing hay content. Hexosamine (mean 36 mg.%) tends to increase with increasing grain concentration. Eighty per cent ethanol soluble dry matter (mean 1.32 mg.%) was greater after feeding. The total nitrogen (mean 37.1 mg.%) was higher on the 20% hay rations and higher after feeding.

P48. Relationships among acetate, propionate, and butyrate metabolism in ruminant liver slices. G. I. Pritchard and S. B. Tove, North Carolina State College, Raleigh.

Acetate, propionate, and butyrate metabolism were studied by incubating C^{14}-labeled substrates with buffered liver slices from a ruminant starved for from six to eight days. Presence of propionate reduced the incorporation of C^{14} from carboxyl-labeled acetate into carbon dioxide, lipids, and protein. Acetate, glucose, or butyrate increased the incorporation of C^{14} from carboxyl-labeled propionate into carbon dioxide, glucose, succinate, malate, and fumarate; whereas, citrate caused no differences. The effect produced by butyrate was greater than that produced either by acetate or by glucose. Apparently, a relationship exists between propionate and butyrate metabolism. This relationship can not be explained on the basis of energy, citric-acid-cycle activity, or competition for the activating enzyme.

P49. Fertility of bull semen stored for one and two days at 5° C. in 20% yolk-citrate-glycine-glucose extenders. R. H. FOOTE AND D. C. YOUNG, Cornell University, AND H. O. DUNN, New York Artificial Breeders' Cooperative, Inc., Ithaca, New York.

In experiments at Cornell, two extenders (CU-16 and CUE) were found to be superior to a 50% yolk-citrate control (C) in maintaining motility of bull spermatozoa. CU-16 consisted of one part of yolk to four parts of buffer containing 1.45% sodium citrate, 1.25% glucose, and 0.937% glycine. CUE consisted of one part of yolk to four parts of a pH-adjusted buffer containing 1.45% sodium citrate, 0.21% sodium bicarbonate, 0.04% potassium chloride, 0.30% glucose, and 0.937% glycine. All extenders contain sulfanilamide, penicillin and dihydrostreptomycin. To test fertility, semen from four Holstein bulls was extended at the rate of ten million motile sperm per milliliter of each extender. Motility studies indicated that there were between eight and nine million motile sperm per milliliter at the time of insemination. Semen was packaged in polystrene tubes. For one-day-old semen the number of first services and the 60- to 90-day nonreturn rate for C was 603 and 78%; for CU-16, 650 and 77%, and for CUE, 601 and 79%. For two-day-old semen the corresponding figures were 373 and 71, 399 and 73, and 386 and 75%.

P50. Prolonged survival of bovine sperm in the Illini variable temperature diluent. N. L. VANDEMARK AND F. D. BARTLETT, JR., University of Illinois, Urbana.

In semen ampuled with the original IVT diluent (*J. Dairy Sci.*, **40**; 438. 1957), sperm motility in split ejaculates from four bulls decreased from 71 to 63% in seven days to 14% after 20 days at 40° F. With higher levels of glucose, egg yolk, and the addition of catalase to the diluent, sperm motility dropped from 71 to 67 and 43% in seven and 20 days, respectively. Further improvement in sperm survival resulted with higher levels of sodium bicarbonate. In storage at 40° F., sperm motility decreased only from 71 to 63% in 20 days. Even at 40 days and 60 days, 57 and 40% actively motile sperm were found, respectively. Storage at 40° F. gave better survival than at 80° F. Fertility of semen stored at room temperature in the original IVT diluent has not been satisfactory in large-scale usage (*Proc. NAAB*, p. 112, 1957). Insemination of 326 cows with semen stored from one through seven days at 40° F. in the IVT diluent, with increased glucose, egg yolk, and added catalase, has resulted in 10–20% higher 60-day nonreturn rates throughout the week over those in the earlier large-scale trials. Fertility tests with semen stored in the diluent which has given even longer sperm survivals are in progress.

P51. A comparison of two diluents in single-service glass ampules for routine artificial insemination of cattle. A. F. MCFEE, L. J. BOYD, AND E. W. SWANSON, University of Tennessee, Knoxville.

The possibility of improving conception rate of fresh liquid semen through four days of age, by providing semen in single-service sealed glass ampules instead of in the common multiple-service plastic vials was investigated. Split ejaculates were diluted in the Illini (IVT) and yolk-citrate (YC) diluents and sealed in glass ampules before cooling. Control YC was also packaged in 5-cc. plastic vials. Cooled semen was shipped from the laboratory to three groups of technicians each day, alternating the two diluents between two of them. Semen was collected from one bull of each of three breeds at four-day intervals for 40 days. Motility at six days averaged 54% in IVT and 57% in YC in ampules. From 160 to 200 services were made each day to each semen treatment. Nonreturn rates (38–78 day) for IVT, YC ampule, and YC vial were, respectively, 75.8, 76.0, and 79.7% at one day; 81.6, 64.0, and 71.1% at two days; 77.7, 51.7, and 64.1% at three days, 62.3, 49.6, and unchecked, at four days. These results indicated *(1)* that there was no advantage to packaging the YC-diluted semen in sealed samples and *(2)* that the ampuled IVT was more efficient than the ampuled YC and YC in vials after the first day's use.

P52. Fertility of bull semen stored at room temperature for six days. H. O. DUNN, New York Artificial Breeders' Cooperative, Inc., AND R. H. FOOTE, Cornell University, Ithaca, N. Y.

In July, 1957, semen from six bulls was extended at a rate of ten million motile sperm per ml. in 50% yolk-citrate at 5° C. and in carbonated Illini Variable Temperature extender at ambient temperature. Approximately 200 technicians made the inseminations with one-day-old control semen shipped in plastic vials and with one- to six-day-old carbonated semen shipped in sealed ampules at ambient temperature.

The 60- to 90-day nonreturn rate for one-day-old semen was 76% for 963 first services. The number of services and nonreturn rates for one- to six-day-old carbonated semen were 152 and 69, 161 and 45, 139 and 33, 121 and 26, 126 and 25, and 139 and 21%, respectively. The total services and average nonreturn rate for the carbonated semen were 838 and 38%. The average nonreturn rates by bull for the control semen were 73, 78, 74, 79, 78, and 77. For the carbonated semen, the corresponding rates were 27, 28, 39, 52, 39, and 45. High ambient temperatures, defects in ampules, and the 2-hr. processing procedure were considered as contributing factors to the low fertility results with the carbonated semen.

P53. Livability of bovine spermatozoa at room temperatures in a coconut milk-citrate-calcium carbonate diluent. CHARLES NORMAN, C. E. JOHNSON, AND I. D. PORTERFIELD, West Virginia University, Morgantown.

A series of metabolic studies performed on three pooled samples of bovine spermatozoa, maintained in a coconut milk-citrate-calcium carbonate medium (1:1 mixture of boiled coconut milk and a 4.32% sodium citrate solution, containing 100 mg. % calcium carbonate, 80 mg. % penicillin, and 90 mg. % dihydrostreptomycin sulfate), indicated that an appreciable amount of metabolic activity could be restored by resuspending the spermatozoa in fresh diluent at the end of six days of storage at room temperatures (23–27° C.). Reactivation of metabolic activity without renewing the diluent was also accomplished by adding $CaCO_3$. Measurements of sperm respiration, anaerobic glycolysis, motility, % live sperm, and pH were made at 3, 76 and 150 hr. after semen collection. Renewal of diluent after 76 hr. of storage showed recovery values of 71, 74, and 83% with respect to sperm O_2 uptake, anaerobic glycolysis, and number of unstained sperm. After six days of storage, renewal of diluent produced recovery of 60, 65, and 84% of the initial values for O_2 uptake, anaerobic glycolysis, and number of live sperm, respectively. Vigorous translatory motility was observed in all reactivated samples.

P54. Comparison of skimmilk-egg yolk-glycerol, egg yolk-citrate-glycerol, and homogenized whole milk-glycerol as extenders for bovine semen. B. W. PICKETT, M. C. JOHNSTONE, W. A. COWAN, AND PAUL HELLER, University of Connecticut, Storrs.

A laboratory study was initiated to compare the storage life of bovine spermatozoa diluted in heated skimmilk-egg yolk-glycerol (A), egg yolk-citrate-glycerol (B), and heated, homogenized whole milk-glycerol (C). The procedure of Sikes and Merilan (1958) was followed for preparation of (A). Concentrations of the skimmilk-egg yolk-glycerol were 82, 10, and 8%, respectively. Split semen samples were diluted in (A), (B), and (C), duplicate samples were stored at 5° C. and at from 0 to −3° C. Results from a limited number of samples observed daily indicated that extenders (A), (B), and (C) maintained a motility rating of one or above (0–4 scale) for 8.5, 5, and 6 days at 5° C., and for 12.5, 5, and 7.5 days at 0 to −3° C., respectively. Samples stored in 2-ml. ampules were superior to those stored in tubes routinely used for shipping liquid semen. Deadlive strains were inconsistent and did not show the differences among extenders that would be expected from the motility ratings. Field trials are in progress to compare the breeding efficiency of Extenders (A) and (B).

P55. Sperm reserves of dairy bulls as determined by depletion trials and postslaughter sperm counts. J. O. ALMQUIST, R. P. AMANN, AND W. T. O'DELL, The Pennsylvania State University, University Park.

Sperm numbers in billions for the combined right and left head, body, and tail of epididymis, ductus deferens, and ampulla of 8 bulls (from 2 to ten years old), sexually rested for 30 days before slaughter, were: 20.3, 4.8, 37.3, 2.4, and 6.1, respectively, totalling 70.9±14.3. In ten bulls depleted just prior to slaughter, the extragonadal reserves, including ejaculated sperm, averaged 69.9±23.0 billion. An average of 37.2 billion sperm was removed immediately before slaughter, representing 53.4±3.6% of the sperm reserves. Thus, depletion trials constitute a reliable estimate of sperm reserves. The 37.2 billion sperm removed agrees closely with the value of 38.7 billion sperm obtained by depleting 27 additional bulls. Depleted bulls contained 23% fewer sperm in the head of the epididymis than rested bulls. There was no increase in the proportion of sperm showing protoplasmic droplets (< 2%) with successive ejaculations. Nonsignificant correlations were found between extragonadal reserves and paired testis weight (±0.247) and paired epididymis weight (+0.329). Testis sperm counts of ten bulls averaged 18.4±4.0 billion per testis, or 56.3±13.6 million per gram of testis.

P56. Sperm production and fertility of dairy bulls at high-collection frequencies with varying degrees of sexual preparation. J. O. ALMQUIST, E. B. HALE, AND R. P. AMANN, The Pennsylvania State University, University Park.

Minimum values for sperm production of three Holstein bulls two, five, and eight years old (based on sperm output when collected 60 to 70 times per week for from 5 to 6 wk. following depletion of sperm reserves) were 7.7, 6.8, and 7.2 billion per day, respectively. Sperm output for three mature bulls collected six times weekly with intensive sexual preparation (including three false mounts for each ejaculate) was 5.1, 6.5, and 7.7 billion per day. These latter values represent an increase of about 30% over output with less intensive preparation (with or without one false mount). Thus, daily sperm output values on six ejaculates per week, collected with adequate sexual preparation, approached the minimum sperm production obtained at very high collection frequencies. With one bull this value was as high as 90% of the estimated sperm production (7.2 billion per day). Observations with bulls collected twice weekly also indicate greater sperm output following three false mounts rather than one. The fertility of three bulls collected six times per week for 8 to 14 mo. was 70, 74, and 77% 60- to 90-day nonreturns to 281, 286, and 656 first-service inseminations, respectively, starting 6 mo. after initiating the six-ejaculates-per-week regime.

P57. Effects of daily ejaculation on sperm output, fertility, and libido of dairy bulls. H. D. Hafs, R. S. Hoyt, and R. W. Bratton, Cornell University, Ithaca, New York.

Semen was collected once a week from six dairy bulls for a 4-wk. preliminary period, after which three bulls were randomly chosen for collecting once a day. During the first 15 wk. of the experimental period, the averages for ejaculate volume, per cent motile, sperm concentration, motile sperm per ejaculate, and total sperm per ejaculate for the bulls collected once a week were 8.37 ml., 64%, 1.68×10^9, 8.93×10^9, and 13.84×10^9, respectively, and for the bulls collected once a day were 5.43 ml., 67%, 0.81×10^9, 3.20×10^9, and 4.60×10^9, respectively. The motile sperm output per week for the daily bulls averaged 22.4×10^9. During this period, daily collection did not measurably affect libido or body weight. For the first 10 wk., the average 28–35 day nonreturn rate was 81.2% for 3,654 first services to the bulls collected once a week and 82.7% for 1,317 first services to the bulls collected once a day. Laboratory studies of the pH, staining properties, and abnormal sperm of the raw semen, and the livability of the sperm during storage at −79° C., 5° C., and room temperature, showed no consistent differences between the two collection frequencies.

P58. Microbiological and chemical changes in grass silage. C. W. Langston, Herbert Irvin, C. H. Gordon, Cecelia Bouma, H. G. Wiseman, C. G. Melin, and L. A. Moore, Dairy Cattle Research Branch, and J. R. McCalmont, Agricultural Engineering Research Branch, USDA, Beltsville, Maryland.

Three cuttings of orchard grass and alfalfa forages ensiled in 4- by 8-ft. experimental silos were treated, to obtain a wide range of quality. Samples were taken from the silos at different stages of fermentation for bacteriological and chemical analysis. The silages were divided into good and poor quality on the basis of pH, ammonia nitrogen, lactic acid, butyric acid, and spore counts. The lactic acid bacteria reached about the same maxima in both the good- and poor-quality forages, and the few silages that showed relatively high initial numbers of lactic acid bacteria were apparently no better in quality than those with few or no lactic acid bacteria. The primary difference in quality in respect to the occurrence of microorganisms was associated directly with the increase in spore-forming anaerobes. All the poor-quality silages showed substantial increases in these organisms. Microorganisms in the good-quality silages produced large quantities of lactic acid, along with fair amounts of acetic and succinic acids. Organisms in the poor-quality silages produced butyric acid at the expense of lactic acid. Acetic, succinic, and propionic acids occurred along with butyric acid.

P59. Preservatives for alfalfa silage. R. J. Baker and H. H. Voelker, South Dakota Agricultural Experiment Station, Brookings.

Third-cutting alfalfa, 75 and 60% moisture, was preserved in glass jars, 60-lb. capacity plastic bags, and 1.5-ton plastic silos. Preservative rates included molasses 4%, lactobacillus plus molasses (?%?), cane sugar 2.4%, sodium chloride 2.5%, ground barley 10%, sprouted barley 10%, ground corn 10%, and no preservative. Total aerobe, proteolytic, lactobacillus, coliform, and pH determinations were made as preservation progressed. The pH dropped fastest and remained lowest (4.3–4.7) in lactobacillus plus molasses, molasses alone, sugar, and sprouted grain silages. Sprouting accentuated pH declines (sprouting should activate amylase, break down starch for quicker lactobacilli use). Salt and control silages were high in pH. Most offensive silage was high-moisture, no-preservative alfalfa in poorly sealed "silos." Forty-two dairy calves were allotted to treatment groups and individually fed ad libitum some of the high-moisture silages from 30 to 148 days of age, testing the feeding values of the preservatives. The rates of consumption and average daily gains (lb.) to date, respectively: sprouted barley first, 1.79; sugar second, 1.91; ground barley third, 1.62; molasses fourth, 1.69; no preservative fifth, 1.66, and salt sixth, 1.52.

P60. Effects of wilting and preservatives on composition of legume-grass silage in miniature silos. R. S. Allen and C. F. Foreman, Iowa State College, Ames.

Nonwilted (69% moisture) and wilted (58% moisture) second-crop alfalfa from the same field were ensiled with no preservative, with added sodium metabisulfite (8 lb/ton), and with added ground corn (100 lb/ton) in concrete miniature silos (2 ft. in diameter, 6 ft. high). Each treatment was replicated. Compaction was accomplished by tramping during filling and thereafter by a constant pressure of 80 lb/sq foot. Samples were taken for chemical analyses at the time of filling and when the silos were emptied from 3 to 3½ mo. later. Wilting resulted in increases (dry matter basis) in amino nitrogen, nonprotein nitrogen, and reducing sugars in the forage as ensiled. Silages resulting from wilted material were substantially lower in amino nitrogen, ammonia nitrogen, and nonprotein nitrogen than the silages from nonwilted forage. Reducing sugar levels were considerably higher, whereas pH values tended to be somewhat lower in wilted silages. Within the group of nonwilted silages, the sodium metabisulfite-treated material contained less ammonia nitrogen and much more (six- to tenfold) reducing sugar than the nonpreserved or corn-preserved silage. Amide nitrogen value were essentially the same in all silages.

P61. Toxic gases from silage. L. C. WANG, R. H. BURRIS, AND R. P. NIEDERMEIER, University of Wisconsin, Madison.

Because newly filled silos may release gases hazardous to livestock and to man, it is important to determine the types of gases produced and the time-course of their production in full-scale silos. Representative samples of silage gases, free of air contamination, were obtained through a fine-bore polyethylene tube supported in a vertical pipe inserted to a height of 5 ft. in a 12- by 40-ft. tower silo filled with corn silage. Periodic analyses of gas samples with a mass spectrometer indicated a rapid rise in the concentrations of CO_2 and NO, a slow rise in N_2O, and a rapid decrease in O_2, N_2, and argon, with time. Mass analysis and colorimetric analysis indicated little production of NO_2 in the silo. This toxic gas is formed from NO when it comes in contact with the oxygen of air. The NO rose to a concentration of ten volume % in the silo gases within 24 hr. and declined to less than 1% within 42 hr. The N_2O continued to rise as the NO disappeared. N_2 disappeared less rapidly than argon, for N_2 was formed by denitrification in the silo. Denitrification can account for considerable losses of nitrogen from silage.

P62. Nutrient losses and feeding values of wilted and direct-cut forages stored in bunker and tower silos. C. H. GORDON, C. G. MELIN, W. C. JACOBSON, H. G. WISEMAN, E. A. KANE, AND J. C. DERBYSHIRE, Dairy Cattle Research Branch, AND J. R. MCCALMONT AND D. T. BLACK, Agricultural Engineering Research Branch, USDA, Beltsville, Maryland.

First-cutting forage, principally orchard grass, was harvested simultaneously from the same fields and stored as *(1)* wilted forage in a tower silo, *(2)* direct-cut forage in a bunker silo, and *(3)* as a direct-cut forage in a tower silo. Both direct-cut forages were treated with 8 lb. sodium metabisulfite per ton. Silage temperatures were recorded for the tower-stored silages. Composition changes, nutrient losses, digestibility, and feeding value for dairy cows were determined for each silage. Top spoilage losses were minimized in all silos by the use of weighted plastic covers. Total dry matter losses were 13.7% in the wilted tower-stored silage, 14.8% in the direct-cut, bunker-stored silage, and 20.9% in the direct-cut, tower-stored silage. Higher seepage losses accounted for a large part of the greater total losses in the latter silage. All silages were of good chemical quality, with pH values of 4.2–4.4. The wilted silage had a higher content of the organic acids measured. Wilted silage dry matter was consumed at the rate of 23.3 lb. per cow daily which, with grain supplement, produced 1.8 lb. of live weight gain and 24.5 lb. of FCM daily. Respective values for bunker-stored silage were 20.1, 0.7, and 25.1, and for direct-cut, tower-stored silage, 20.5, 1.3, and 23.5.

P63. Feeding value for milking cows of oat silages cut at three stage of maturity. F. A. MARTZ, C. H. NOLLER, D. L. HILL, AND M. W. CARTER, Purdue University, Lafayette, Indiana.

The feeding value of oat silages cut at three stages of maturity—*(1)* boot, *(2)* milk, *(3)* dough—and *(4)* alfalfa-ladino-brome grass silage were compared. The silages were direct-cut with 6–8 lb. sodium metabisulfite per ton and stored in upright silos. Twelve lactating dairy cows were used on three 4×4 Latin-squares, with an extra period to measure carry-over effect. A seven-day preliminary period was followed by an eight-day experimental period. The silages were fed ad libitum plus 0.3 lb. hay daily per 100 lb. body weight and an 18.9% crude protein grain at 1:6. The silages contained 79.0, 76.5, 70.1, and 74.5% moisture and had a pH of 4.40, 4.65, 4.60, and 4.39, respectively. The titratable acidity was 0.79, 0.56, 0.84, and 1.11%. The treatment means for dairy FCM were 23.3, 21.1, 22.2, and 21.7 lb., respectively. The silage treatments were statistically significant ($P \leq 0.05$). The average daily silage dry matter intakes were 21.9, 21.0, 25.4, and 19.9 lb., respectively. The dry matter intakes were significantly different ($P \leq 0.01$). The average body weight changes per cow per 15-day period were −2.5, 0.0, +10.5, and −4.5 lb., respectively. There were no apparent carry-over effects.

P64. Soilage or silage for lactating dairy cows? C. F. FOREMAN, R. S. ALLEN, AND A. R. PORTER, Iowa State College, Ames.

A comparison was made between first-crop alfalfa silage (bunker silo, 10 lb. sodium metabisulfite/ton) and second-crop alfalfa (chopped and fed fresh daily), when fed to lactating cows. Grain was fed at a constant level, based on individual milk production during the 2-wk. pre-experimental period on pasture. Average pounds daily milk production, per cent total solids (TS), and per cent butterfat (BF) during the pre-experimental period were 45.7, 11.29, 3.10, and 45.6, 10.87, 2.87, for cows on soilage and silage, respectively. Average pounds daily milk production, % TS, and % BF during the 8-wk. experimental period for soilage- and silage-fed cows were 42.3, 11.50, 3.03, and 36.5, 11.10, 3.04, respectively. Most of the decrease in milk production on silage occurred during the 1st wk. of the experiment. The mean per cent protein, crude fiber, and N.F.E. on a dry basis was 20.55, 26.04, and 42.41 for soilage, and 17.44, 31.85, and 35.85 for silage. A percentage increase in crude fiber, and a decrease in protein and N.F.E. in the silage, occurred as the experiment progressed. Variations in milk production and % TS, and in the composition of soilage and silage, will be discussed.

P65. Milk production correlated with quality and quantity of daily herbage intake. A. L. Brundage and W. J. Sweetman, Alaska Agricultural Experiment Station, Palmer.

Six lactating dairy cows were used during the grazing season of 1955, to study the correlation of herbage intake and milk production under rotational and strip grazing. Fecal samples were obtained at 7:30 A.M. and 4 P.M. daily and composited into consecutive three-day samples in the manner of a running average. The combined Cr_2O_3-chromogen procedure was employed, to compute apparent dry matter digestibility and total intake. Multiple correlation analyses were computed for each cow, using time, digestibility, fecal output, digestible intake, bi-daily variation in liveweight, and milk production as variables. Under strip grazing, nearly all the variation in daily milk production was attributed to time. Time accounted for less of the variation in milk production under rotational grazing. A highly significant portion of the remaining variation was attributed to fluctuation in digestibility and digestible intake. These data suggest that digestibility and digestible intake are limiting factors under rotational grazing but not under strip grazing.

P66. Forage and milk yields from alfalfa under three different harvesting systems. R. M. Porter and S. R. Skaggs, New Mexico Agricultural Experiment Station, State College.

The experiment was set up to determine the relative yield of forage, labor costs, and milk yield when alfalfa was used for pasture, soiling crop, or soilage. A 10% increase in yield was obtained by mechanical harvesting. No bloat problem was experienced using the California system of controlled grazing, and excellent consumption of available forage was obtained. There was no significant difference in milk yield while utilizing alfalfa as pasture, silage, or soiling crop. Pasturing of alfalfa was the most economical, but it is felt that this method would have limited use.

P67. Fertility of frozen semen stored in plastic and glass ampules and of first and second ejaculates. H. H. Brugman and R. P. Schmidt, University of Maine, Orono.

The Connecticut and Massachusetts Artificial Breeding Cooperatives supplied the semen. Each ejaculate was split and half was placed in glass ampules and the other half in plastic 1-cc. polybulbs. Connecticut discontinued the use of polybulbs because they floated. Connecticut had 235 first services. Sixty–ninety day nonreturn for the first ejaculate was 48.0% and for the second was 57.4%. Massachusetts had 492 first services. Sixty–ninety day nonreturn for the first ejaculate (glass and plastic) was 50.2%; for the second ejaculate (glass and plastic), 65.4%, both ejaculates in plastic, 59.9%, and in glass, 56.0%. The difference in conception rate between first and second ejaculates was highly significant ($P < 0.01$). Difference between plastic and glass ampules was not significant ($P > 0.05$). This study indicates that plastic polybulbs or glass ampules can be used satisfactorily for storing frozen semen in CO_2 and alcohol. Polybulbs are difficult to store because they float. Conception data showed a highly significant difference in favor of the second ejaculate.

P68. Addition of progesterone and estrogen to bovine semen. R. E. Staples, W. Hansel, R. H. Foote, and H. O. Dunn, Cornell University, Ithaca, New York.

Progesterone (1–10,000 μg. per ml.) and estradiol (0.01–800 μg. per ml.), alone and in combination, were added to bovine semen extended with yolk: citrate to ten million motile sperm per milliliter. No differences attributable to hormone treatments were observed in motility or longevity of the semen during storage at 5° C. Two fertility trials were conducted which involved a total of 3,245 dairy cows and eight dairy bulls. In the first trial, 20 μg. of progesterone and 1 μg. of estrogen per milliliter of extended semen were used, both singly and in combination; in the second, 5,000 μg. and 200 μg. per ml. were used, respectively. The 60–90 day per cent nonreturns in the first trial were as follows: Control—75, progesterone—73, estradiol—74, and progesterone plus estradiol—73. In the second trial, the 60–90 per cent nonreturns were: Control—70, progesterone—71, estradiol—72, and progesterone plus estradiol—69.

P69. Density of "sampler" bulls. R. B. Becker and P. T. Dix Arnold, Florida Agricultural Experiment Station, Gainesville.

Records of 1,002 dairy bulls started in artificial use between 1942 and 1951 as yearlings and 2-yr.-olds, were analyzed, to determine the average fate of so-called sampler bulls. Of the 317 bulls culled, low production of daughters accounted for 40%; poor conformation, 22%; replaced by better bull, 18%; unpopular, 10%, and the remaining 10% for bad disposition, inbreeding, expiration of lease, stud disbanded, or never used. The average age at culling was 5.5 yr. old. One bull was 1.1 yr. old, and ten bulls were past 10 yr. old before this final decision was made. Low breeding efficiency terminated use of 48% of 460 bulls removed from artificial use by natural causes and some before they had daughters in milk. Of these 1,002 bulls, 32% proved unsatisfactory; 46% left service for natural causes at various ages, and the remaining 22% were still in use when from 6 to 13 yr. of age.

P70. Results from breeding cows with frozen semen thawed for various lengths of time. Victor Hurst and J. W. Kelly, South Caro-

lina Agricultural Experiment Station, Clemson.

During the spring and summer of 1957, heifers and cows in the Clemson dairy herd were inseminated with frozen semen after it had been thawed for different periods of time. Results of breedings based on pregnancy examinations at 3 mo. were as follows: Semen used within 0.5 hr. after thawing, 54 inseminations, 43% pregnant; semen used 1 hr. after thawing, 48 inseminations, 38% pregnant; semen used 2 hr. after thawing, 13 inseminations, 31% pregnant; semen used 3 hr. after thawing, five inseminations, 20% pregnant; semen used 5 hr. after thawing, one insemination, 100% pregnant. By eliminating low-conception bulls (below 33% on pregnancy diagnosis) the conception rates in the 0.5-, 1-, and 2-hr. groups were raised to 51, 52, and 60%, respectively.

P71. Some aspects of the role of glycerol in the metabolism of bovine semen. R. J. Flipse and A. A. Benson, Pennsylvania Agricultural Experiment Station, University Park.

Bovine spermatozoa were washed free of seminal plasma, then incubated aerobically with glycerol-1-C^{14} in Ringer-phosphate. CO_2 was collected during incubation; after the 3-hr. incubation period the cells were washed free of the medium and extracted, first with 0.6 N perchloric acid (PCA), then with 95% ethanol, and finally with ethanol-ether. Of the recovered radioactivity, 65% was in CO_2, 8% in the PCA extract, 22% in the combined ethanol and ethanol-ether extracts, and 5% in the residue after extraction. The PCA-soluble fraction was paper-chromatographed and most of the activity was attributed to lactate, although radioactivity also was noted in succinate and in several unidentified compounds. Components of the phospholipids of bovine spermatozoa were characterized by activation analysis. Phospholipids were extracted from plasma-free spermatozoa, hydrolyzed with methanolic KOH, and the hydrolysate chromatographed on paper. After two-dimensional development, the chromatogram was irradiated at a flux of 5×10^{11} neutrons/cm^2/sec for 5 hr. The radioactivated phosphorus was then used to locate glycerophosphate esters. By this procedure, glycerophosphorylcholine, glycerophosphorylethanolamine, glycerophosphorylserine, and diglyceerophosphate were identified as derivatives of sperm phospholipids. The function of these esters in the metabolism of spermatozoa will be discussed.

P72. Comparison of filtered versus nonfiltered bovine semen after extension and low-temperature storage. M. A. Brown and J. C. Johnson, A & M College of Texas, College Station.

An apparatus utilizing pima-cotton broadcloth as a filter allowed, on the average, 32% of spermatozoa to pass through the filter. Nonfiltered control portions of split ejaculates contained an average of 59% live sperm, whereas filtered aliquots of the samples contained an average of 77% live cells. The filter was effective in removing a portion of the dead and abnormal spermatozoa from bovine semen samples. Extended semen, from which an appreciable portion of dead and abnormal cells had been removed, had an average survival rate of 20% after glycerolization, equilibration, and storage at −79° C. for seven days. Nonfiltered semen similarly treated had an average survival of 39%. It appears that one or more semen components that contribute to improved sperm livability during low-temperature storage may have been removed, at least in part, by the filtering process.

P73. Alanine-pyruvate conversions in bovine semen. R. J. Flipse and W. R. Anderson, Pennsylvania Agricultural Experiment Station, University Park.

Previous work in this laboratory has shown that labeled glyoxylate is produced when spermatozoa are incubated with glycine-C^{14}. This has prompted investigation of the possibility that other transaminations may occur in these cells. A pyruvate-alanine system would be of particular interest. Lactic acid accumulates as a result of sperm metabolism, but the accumulation is less in glycine-containing diluents than in milk or egg yolk–citrate. Sperm extracts were prepared by mechanically disrupting the washed cells, centrifuging off cellular debris, and dialyzing the extract against phosphate buffer. Incubations were conducted at 37° C. with nitrogen as the gas phase. Each system included glycine, glyoxylate, pyridoxal phosphate, and *(a)* dialyzed sperm extract and alanine-1-C^{14}, *(b)* dialyzed sperm extract and pyruvate-2-C^{14}, *(c)* dialyzed seminal plasma and alanine-1-C^{14}, or *(d)* washed spermatozoa and alanine-1-C^{14}. In each of the systems tested, evidence was obtained of alanine-pyruvate conversion in excess of that obtained in the absence of extract or with heat-treated extract.

P74. Investigation of motility of sperm thawed at different temperatures and time intervals. H. H. Brugman, M. E. Poore, and R. P. Schmidt, University of Maine, Orono.

The Breeding Cooperatives in Maine, Massachusetts, and Connecticut supplied semen for this experiment. Semen was extended at the cooperatives in 20% egg yolk–citrate and 7% glycerol. Equilibration took place in transit to the Orono laboratory, and the time involved approximately 18 hr. Thirty-seven Holstein and Guernsey bulls were used. Two hundred and thirty samples from 3,160 ampules were analyzed. There was no significant difference in motility between thawing temperatures of 5 and 38° C. at intervals of 3, 6, 24, and 72 hr. Variation between studs was highly signifi-

cant ($P < 0.01$). Variation between breeds and between bulls within studs was also highly significant ($P < 0.01$).

P75. Recovery of sperm from the exteriorized bovine uterine cervix. H. D. Hafs, D. T. Armstrong, and R. W. Bratton, Cornell University, Ithaca, New York.

The cervix of each of five heifer calves ranging in age from 1 to 21 wk. was relocated to the region of the left flank by a surgical technique similar to that used by Amantea in the dog and Reynolds in the rabbit. Following surgery, the heifers grew normally and there was no evidence of infection in the exteriorized cervix. Starting at 17 mo. of age, the mean length for 55 estrous cycles in four heifers was 20.3 days, with a standard deviation of 4.27 days, omitting one heifer which developed a cystic follicle. Ten billion nonmotile sperm were injected into the estrous uterus of each of the four heifers at each of nine successive estrous periods. After three incubation periods in each heifer, of either 0, 5, or 10 hr., an average of 49, 15, and 6%, respectively, of the injected sperm were recovered by flushing the uterus with two 250-ml. portions of saline. Three attempts to impregnate each heifer have failed. However, the relatively large number of sperm that may be recovered from the uterus by this surgical preparation offers an opportunity to study more extensively the effects of the uterine environment on sperm, particularly on their maturation.

P76. Effects of an intrauterine infusion of penicillin and streptomycin on reproductive performance in dairy cows. J. M. Buckalew and J. A. Alford, Mississippi State College, State College.

For 2 yr., 89 animals have been given an intrauterine infusion of 500,000 units of penicillin and one gram of streptomycin in 20 ml. of sterile water during the estrous period prior to the one in which the cow was to be bred. A group of equal size served as a control. Cows that showed evidence of severe postpartum metritis, or that had a history of difficult breeding, were not used in the study. Bacterial swab samples were taken from the uterus during estrus and luteal phases prior to breeding, and during any subsequent estrous periods. To date, the treated cows have required 1.3 services per conception, as compared to 1.8 for the control group. Twenty-one of the control and five of the experimental animals have required three or more services to conceive. Bacterial examination revealed a wide variety of organisms present in the genital tract during all phases of the estrous cycle. No particular organism was found to be associated with the repeat-breeding cows, and no specific effect of the antibiotics on the uterine flora was revealed.

P77. Effects of oxytocin on bovine ovarian function. D. T. Armstrong and William Hansel, Cornell University, Ithaca, New York.

It has been shown that seven daily injections of oxytocin (free of contaminating gonadotropins), beginning on the day of estrus and prior to ovulation, consistently cause normally-cycling dairy heifers to return to heat and to ovulate from one to four days after injections are stopped (Armstrong and Hansel, *Fed. Proc.*, in press). Further studies have shown that even if oxytocin injections are not discontinued, heifers return to heat in approximately nine days afteh the previous estrus. This indicates that the return of estrus is not owing to oxytocin withdrawal, but more likely depends upon its action over a period of time. Four daily injections were insufficient to induce estrus. Gross and histological examinations of ovaries at various times during oxytocin treatment have revealed corpora lutea of smaller than normal size which, in some cases, contained fluid-filled cavities or cysts. The induction of estrus and ovulation by oxytocin could not be prevented by concurrent injections of prolactin, but could be prevented by the anticholinergic drug, atropine. These experiments are being continued, in an effort to elucidate the mechanism of this action of oxytocin and to determine if this method might be useful in controlling estrus and ovulation artificially.

P78. Morphogenesis and histochemistry of the bovine placenta between 16 and 33 days of gestation. J. S. Greenstein, R. W. Murray, and R. C. Foley, University of Massachusetts, Amherst.

Preliminary standards of daily development for the bovine placenta from 16 to 33 days of pregnancy have established, based on material recovered from 84 heifers and cows slaughtered at known intervals after insemination. The proliferating trophoblast gives rise rapidly to two distinct cell types characteristic of later stages: the ordinary trophoblastic columnar cells and the typical ruminant giant cells. The former appear to serve both a nutritive and secretory function. The latter are recognizable as early as 16–17 days, and their histochemistry suggests that they may play a role in the maintenance of the corpus luteum of pregnancy. The placenta contains only minimal amounts of glycogen, is rich in neutral fats, and a carbohydrate-protein complex is concentrated in the giant cell cytoplasm. The temporary yolk sac and vitelline circulation are gradually reduced in size, and function with the emergence on day 20 of the expanding allantois, resulting in the formation of a vascularized chorioallantois by day 27. Chorionic villous projections appear on day 31, preparatory to placentation. The successful development of the yolk sac, giant cells, vitelline circulation, and chorioallantois are all considered to be critical stages for embryo survival.

P79. Survival of rabbit ova stored at −79° C. MANSUR FERDOWS, C. L. MOORE, AND A. E. DRACY, South Dakota Agricultural Experiment Station, Brookings.

Fertilized rabbit ova were stored at −79° C. The eggs were equilibrated at 10-min. intervals with a one-fifth volume of 20% glycerol saline solution, so that a 10% glycerol solution resulted and was stored in a refrigerator until the temperature dropped to 2° C. The first temperature drop from 2 to −15° C. was carried out at the rate of a one-degree drop in temperature per each 10 min. The second step, from −15 to −35° C., was carried out at the rate of a one-degree drop in temperature per each 5 min. From −35 to −79° C., the temperature drop was one degree per each 2.5 min. At the end of this time, the ova were stored in a dry-ice chest until ready for transplantation. The ova were thawed at room temperature and transplanted into a doe, previously prepared for conception either by the injection of gonadotropin or by copulation with a vasectomized male. Sixty-five ova were transplanted into eight does. Of the eight does, two showed pregnancy—one with eight fetuses and one with ten developed fetuses—thus suggesting the possibility of storing ova at −79° C.

P80. Relationships among duration of estrus, ovulation time, time of service, and fertility of dairy cattle in Louisiana. J. G. HALL, C. BRANTON, E. J. STONE, AND R. B. LANK, Louisiana State University, Baton Rouge.

Studies of duration of estrus, ovulation time, time of service, and fertility in the L.S.U. dairy herd have revealed that recommendations to inseminators and farmers should be different from those for the temperate regions of the country. The average duration of estrus for 1,055 periods on 242 cows was 12.37 hr., ranging from 2 to 40 hr. During 10.05% of these periods, the cows failed to show standing heat. There was no apparent seasonal variation in duration of estrus. Little variation was found among the Holstein-Friesians, Jersey and Sindhi-Holstein crossbreds. The average number of days to first postpartum estrus was 31.95. The average interval between estrous cycles was 20.97 days. Only a limited number of cows were palpated to determine ovulation time. These data gave an average of 12.80 hr. from the end of estrus. These determinations are being continued. Conception rates for cows bred at 0–6, 7–12, 13–18, and 19–24 hr. after the first sign of estrus were 37.5, 58.5, 40.2, and 34.8%, respectively. Conception rate is being defined as diagnosed pregnancies to the first three services. There has been a total of 250 services to date.

P81. Bactericidal activity of inflammatory exudes from rabbit uteri. H. W. HAWK AND J. F. SYKES, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Bactericidal substances in inflammatory exudate may play a part in the endocrine-controlled uterine defense mechanism. Exudate from estrous rabbit uteri inoculated 4 hr. previously is highly bactericidal, and few bacteria remain alive in the uteri; pseudopregnant rabbit exudate is only slightly bactericidal at 4 hr., and the uteri contain many bacteria at various intervals after inoculation. This could be owing to a continued lack of bactericidal substances in pseudopregnant rabbit uteri. Therefore, a test was made of the bactericidal activity of exudates at a later stage. Each uterine horn of eight pseudopregnant and eight estrous rabbits was inoculated with *Escherichia coli*. Exudate was collected from one horn 16 hr. later; the number of live bacteria was determined in the other. Leukocyte-free exudates and saline were inoculated with equal numbers of *E. coli* and incubated at 37° C. for 2 hr.; mean numbers still alive were: pseudopregnant rabbit exudate, 2,750; estrous rabbit exudate, 2,450,000, and saline, 117,000,000. Exudate of pseudopregnant rabbits is thus extremely bactericidal at 16 hr., even more so than estrous rabbit exudate ($P < 0.01$); despite this, more *E. coli* were alive in the pseudopregnant than in estrous rabbits. Factors other than a lack of bactericidal substances in pseudopregnant rabbit uteri must account for this difference.

P82. Effect of synthalin A on renal function in dairy bull calves. R. R. ANDERSON AND J. P. MIXNER, New Jersey Agricultural Experiment Station, Sussex.

Synthalin A is a toxic, hypoglycemic agent which has been reported to cause histological changes in the liver, kidneys, and alpha cells of the Isles of Langerhan, and also to depress hepatic function in dairy bull calves. Five Holstein bull calves (eight days of age) were injected intramuscularly with Synthalin A at the rate of 2.2 mg. per kg. of body weight. Plasma glucose levels decreased from a mean preinjection level of 85.6 to 32.8 mg. % 16 hr. after injection, and returned to normal levels again within 66 hr. after injection. Renal function was measured by the phenolsulfonphtalein (PSP) fractional clearance test. PSP fractional clearance decreased from a mean preinjection level of 0.0273 to 0.0102 within 16 hr. after injection (37.4% of normal), and returned to 81.0% of normal within seven days. The effects of Synthalin A in decreasing both plasma levels of glucose and PSP fractional clearance were highly significant ($P < 0.01$). Recovery of renal function was somewhat more delayed than the return to normal of plasma glucose levels, following the Synthalin A injections.

P83. Relationships between plasma protein-bound iodine and certain measures of reproductive and lactational performance in dairy cattle. H. D. LENNON, JR., AND J. P. MIXNER, New Jersey Agricultural Experiment Station, Sussex.

Plasma protein-bound iodine values were obtained at 60 days postpartum on 93 Holstein cows (139 lactations) and 34 Guernsey cows (57 lactations), over a 3-year period, and these values were correlated on a within-lactation basis, with certain concurrent measures of reproductive and lactational performance. Measures of reproductive performance included length of interval from parturition to conception, length of interval from first breeding to conception, and number of services per conception. Measures of lactational performance included 240-day 4% FCM production, maximum initial milk yield, persistency index, per cent fat, and per cent solids-not-fat. The Holstein data gave significant or highly significant negative correlations between the levels of plasma protein-bound iodine and the interval from parturition to conception (-0.255^{**}), interval from first breeding to conception (-0.251^{**}), services per conception (-0.213^{*}), and per cent fat (-0.179^{*}). No other correlations in either breed reached the level of statistical significance.

P84. Effect of pregnant mare serum on the sexually immature bovine testis.* ENRIQUE SANTAMARINA AND R. P. REECE, New Jersey Agricultural Experiment Station, New Brunswick.

Data are not available concerning the effect of gonadotrophic hormones on the testis of immature bovines; furthermore, hormonal therapy in the bull is based mainly on the limited information obtained from clinical data. An attempt has been made, by a study of the histological elements of the testis, to determine at what age the bovine testis becomes responsive to endogenous gonadotrophic hormones. Pregnant mare serum was administered to nine Jersey and Brown Swiss bull calves. Hormonal treatment was started when the calves were from 12 to 93 days of age. The total dosage ranged from 800 to 25,600 I.U. The diameter of the seminiferous tubules, the differentiation of the germinal cells, spermatogenesis and, especially, the maturation of the interstitial tissue were stimulated in some degree in every case. The interstitial tissue, however, was more sensitive to PMS than the other histological elements of the testis.

* Supported by a grant from the U. S. Public Health Service (RG—4726).

P85. Some causes of variation in estrual cycle length in Holstein-Friesian heifers. S. E. MARES, A. C. MENGE, W. J. TYLER, AND L. E. CASIDA, University of Wisconsin, Madison.

Effects of inbreeding, season of birth, infertile mating, and individuality have been determined on the lengths of 957 estrual cycles of 221 Holstein-Friesian heifers. Infertile inseminations were made at the beginning of 191 of the cycles and they will be referred to as return-intervals. The remaining estrual cycles had a mode of 21 days and a range of from three to 102 daays. The corresponding values for the return intervals were 20 and 4 to 92 days, respectively. There was a higher percentage of return intervals than of estrual cycles in the 24–33 day range and a lower percentage in the 19–23 day range. The intra-cow correlation for estrual cycle lengths (transformed to logarithms) within sire-lines and systems of mating was 0.096 ($P < 0.01$), and for return interval lengths, 0.062 ($P > 0.05$). Variances within cows differed among the different sire-lines, but did not differ significantly between outbred and inbred heifers, whether from outbred or inbred parents. Length of estrual cycles was affected by both season of birth and system of mating. The longest cycles were shown by spring-born, inbred heifers from inbred parents.

P86. Age at puberty in Holstein-Friesian heifers. A. C. MENGE, S. E. MARES, AND L. E. CASIDA, University of Wisconsin, Madison.

Effects of system of mating, growth rate, season of birth, and calfhood scours on age at puberty were studied in six sire-lines involving 184 heifers. Each sire-line was represented by outbred heifers from outbred parents, inbred heifers from outbred parents, inbred heifers from inbred parents, and outbred heifers from inbred parents (line cross). Significant differences existed among the sire-lines for age at puberty. System of mating also had a significant effect on age at puberty, with line-cross heifers attaining puberty at an earlier age than the inbreds from outbred parents ($P < 0.01$).

Six-month weight was 38 lb. less in heifers which had scoured as calves, compared to the nonscouring heifers ($P < 0.01$). Age at puberty had regressions of -0.77 days on 6-mo. weight and -0.36 days on weight gain from 6 to 12 mo. ($P < 0.01$ and $P < 0.05$, respectively). Scouring caused a delay of puberty only in the fall-born heifers. This delay of 136 days ($P < 0.01$) could not be accounted for by the effect of scouring through growth rate alone.

P87. Genetic improvement of dairy cattle attributable to sires used in artificial insemination in North Carolina. W. L. TUCKER AND B. R. FARTHING, North Carolina State College, Raleigh.

This study was initiated to evaluate the genetic advantage which actually has been realized in North Carolina from sires used in artificial insemination. The milk production, fat production, and fat test of 6,988 2×, mature

equivalent records of Holstein, Guernsey, and Jersey cattle were studied. The effect of season of calving on production was pronounced. Cows freshening during the months of December through May produced 21.0 lb. of fat and 595.0 lb. of milk more than cows freshening in June through November. First lactations of artificially sired daughters were compared with the first lactations of naturally sired animals freshening in the same herd, season, and year. The artificially sired daughters produced 15.7 lb. of fat and 366.0 lb. of milk more than their naturally sired contemporaries. The bias introduced by the daughter-dam comparison and the contemporary comparison, using all naturally sired contemporary records, was studied. Environmental differences, as well as selection of dams, biased the results from the daughter-dam comparison. When all naturally sired contemporary records were used for the contemporary comparison, the environmental bias was eliminated, but selection of animals which produced later records biased the results.

P88. Withdrawn.

P89. Heritability and repeatability of some blood constituents of dairy cattle. MOGENS PLUM AND A. B. SCHULTZE, University of Nebraska, Lincoln.

Blood samples of 50 heifers were analyzed at monthly intervals over a 10-mo. period beginning at 3 mo. of age. The blood samples were analyzed for hemoglobin, red blood cell count, circulating eosinophils, and blood sugar. The 50 heifers were sired by 12 different sires. Using averages of ten determinations, the heritability estimates obtained by paternal half-sister correlations were hemoglobin, 0.5, eosinophils, 0.6, red blood cell count, 0.7, and blood sugar, 0. The repeatabilities of determinations were as follows: blood sugar, 0, red blood cells and eosinophils, 0.1, and hemoglobin, 0.2. Heritability estimates based on single determinations are considerably lower than the ones determined by averages of ten analyses, viz., 0.20 for red blood cell count and 0.14 for hemoglobin and eosinophils.

P90. Comparison of a commercial milk-replacer ration with whole milk in a calf-raising system. M. W. NEATHERY, M. E. MCCULLOUGH, O. E. SELL, AND L. R. SISK, Georgia Mountain Experiment Station, Blairsville.

Thirty, four-day-old Guernsey calves were assigned equally, according to birth weight, to the following five rations: 1, whole milk; 2, milk replacer; 3, whole milk plus vitamin B_{12}; 4, milk replacer plus vitamin B_{12}, and 5, equal mixture of milk replacer and skimmilk. Liquid rations were fed daily at the rate of 10% of birth weight from four to 42 days of age. The carry-over effect of these rations was measured for an additional 42 days and a grain mixture and orchard grass hay were fed throughout the experiment. Weight gains from the whole milk rations (1 and 3) were significantly greater than gains from the milk-replacer rations (2, 4, and 5). Performance of both types of rations was increased, but not significantly, when vitamin B_{12} was added. Addition of skimmilk to the milk-replacer ration (2) did not significantly increase weight gains. The calves that received the whole milk rations continued to grow at a significantly higher rate during the carry-over period. Feed efficiency was significantly greater for calves receiving the whole milk rations. Some loss of hair was observed on two-thirds of the calves on the milk replacer ration (2). Scours was more prevalent among calves on the milk-replacer rations.

P91. Comparison of alfalfa hay, alfalfa silage, and corn silage as sole roughages for young dairy calves. K. E. GARDNER, University of Illinois, Urbana.

Twenty-one new-born calves were raised to 16 wk. of age on ad libitum feeding of one of three roughages—alfalfa hay, alfalfa silage, or corn silage. A total of 350 lb. whole milk was fed and a 19% T.P. starter was fed ad lib. up to 4 lb. daily. Gains averaged 154, 146, and 148 lb. daily on the alfalfa hay, alfalfa silage, and corn silage groups, respectively. When the effect of birth-weight differences was considered, the differences in gains were not significant. Average consumption of roughage was 179, 317, and 353 lb. for the three groups as listed. Starter intake was 275, 293, and 259 lb., respectively.

P92. Utilization of several fats by dairy calves. D. T. HOPKINS, R. G. WARNER, AND J. K. LOOSLI, Cornell University, Ithaca, New York.

As part of a study to determine satisfactory methods of adding fat to milk replacers, the following experiment was conducted: Different fats were mixed, at a level of 20%, with a basal diet of spray-dried skimmilk, Vitamin A, irradiated yeast, and Aurofac D. These diets were mixed with ten times their weight of warm water and fed to male Holstein calves. The fats used were (1) tallow (M.P. 40.0 C°), (2) cocoanut fat (M.P. 24.4 C°), (3) butter (M.P. 30.7° C.), (4) tallow and crude soybean lecithin, and (5) cocoanut fat and crude soybean lecithin. In addition, at the time of feeding, a homogenate of cocoanut fat, crude soybean lecithin, and water was added to the basal diet (6). Between the ages of 2 and 4 wk. digestion trials were conducted, with a minimum of four trials per diet. Feed was analyzed for ether extract; feces were analyzed for ether extract and soaps. The following digestion coefficients for total fat were obtained: (1) 59.2, (2) 65.4, (3) 67.3, (4) 72.1, (5) 95.6, and (6) 94.9. The coefficients for (5) and (6) were

significantly higher than those for the other diets. That for (4) was significantly higher than (1).

P93. Tetraalkylammonium stearate in a milk replacer and a dry calf starter. W. A. Olson and J. B. Williams, University of Minnesota, St. Paul.

Twenty-four calves were used in a 2^2 factorial experiment, to study the effects of tetraalkylammonium stearate in *(1)* a reconstituted dry skimmilk ration and *(2)* a calf starter containing 20% ground alfalfa hay, 25% ground corn, 20% soybean oil meal, 15% wheat bran, 12.5% ground beet pulp, and 2.5% vitamin and mineral supplement. Tetraalkylammonium stearate was added to the milk replacer to supply 200 mg. per calf per day, and to the calf starter at the rate of 40 mg. per pound for those animals receiving the treatment. Two Jersey bulls, six Holstein heifers, and 16 Holstein bull calves were employed from the fifth through the 42nd day of age. Whole milk was fed as one-half of the milk ration until 11 days of age. Spray-process nonfat dry milk was reconstituted 1:10 with water and fed at approximately 10% of the calves' body weight daily. The milk replacer was fed at 100° F. in open-top pails. The calf starter was offered ad libitum to the calves after the fourth day of age. Water was available to the calves at all times. Weight gains were similar with all treatments. Occurrence of scouring was greater and starter consumption was not as great, with the animals treated with tetraalkylammonium stearate, in both milk replacer and starter. There was no great difference in calf body-temperature between groups for the first 2 wk. of life.

P94. Effects of feeding volatile fatty acids to young calves. W. G. Martin, H. A. Ramsey, G. Matrone, and G. H. Wise, North Carolina State College, Raleigh.

Nine calves (three replications), starting at three days of age, were fed the following diets for 12 wk.: Diet I: casein, fat, glucose, starch, and chopped hay; Diet II: casein, fat, glucose, and salts (31.9% of diet) of acetic, propionic, and butyric acids; Diet III: whole milk. Average daily gains (lb.) were: I, 0.79; II, 0.93, and III, 1.15. Samples of venous blood (0, 3, and 6 hr. after feeding) and of urine were taken weekly and analyzed for glucose, ketones, and volatile fatty acids (VFA). After feeding, blood glucose increased sharply for Diet III but decreased slightly for I and II. Conversely, blood ketones and VFA increased markedly for Diets I and II but did not change for III. For Diet II, blood VFA were highest at 3 hr. after feeding, and by 3 wk. of age, such values were similar to those found in the adult ruminant. Only small differences among diets were noted for any of the blood constituents immediately before feeding. At 0-hour, blood glucose tended to decrease with increasing age, whereas VFA and ketones exhibited no marked change. Calves on Diet II excreted the largest quantities of urinary ketones and VFA, whereas the least quantities of urinary glucose were excreted on Diet I. In general, the total excretion of all urinary constituents tended to increase with increasing age.

P95. Effects of acetate supplementation in calf feeding. J. R. Staubus, R. E. Brown, C. L. Davis, and W. O. Nelson, University of Illinois, Urbana.

In a preliminary study, six Holstein bull calves were fed milk at the rate of 10% of body weight, plus starter free choice, to 12 wk. of age. In addition to the basal ration, three of the calves were fed 50 g. of sodium acetate daily in the milk. The average blood glucose concentrations at 12 wk. were 95.3 and 89.7 mg/100 ml., as compared to initial levels of 98.1 and 110.6 mg/100 ml. for the acetate-fed and control calves, respectively. It is of interest that no pronounced drop in blood glucose occurred in either group. Acetate supplementation did not result in increased blood VFA levels. Rumen development as measured by wet weight, ratio of rumen and reticulum to abomasum, and papillary development varied between calves but not between groups. The most marked effect of acetate supplementation was in rate of growth and feed efficiency. The acetate-supplemented calves grew at a rate of 1.90 lb/day with a feed efficiency of 1.47 lb. D.M/lb of gain. The respective values for the control calves were 1.45 lb/day and 1.61 lb. D.M/lb of gain. Data from a more extensive trial involving 18 calves will be presented.

P96. Effect of a mixture of chlortetracycline, bacitracin, and penicillin on dairy calves. L. L. Rusoff and W. J. Milstead, Louisiana Agricultural Experiment Station, Baton Rouge.

Twenty-four male Holstein calves were divided at random into three groups: Group I served as the control; Group II received 50 mg. of a mixture of three antibiotics (25 mg. of CTC, 18.75 mg. of zinc bacitracin, and 6.25 mg. of procain penicillin) daily, and Group III received 50 mg. of chlortetracycline daily. Colostrum was fed for the first three days, then whole milk at the rate of 10% of body weight up to 30 days of age. A plant-protein calf starter and medium-quality hay were fed beginning at four days of age. The calf starter for Group II was supplemented with a 1% supplement mixture of the three antibiotics (0.92 g. CTC, 0.72 g. bacitracin, and 0.24 g. of penicillin), and the calf starter for Group III, a 1% supplement of CTC (1.86 g. CTC per lb.). At 8 wk. of age, both antibiotic-fed groups (II and III) showed increased gains in weight by 15%, increases in height at withers, and increased

feed consumption over those of the control group. Hay consumption by the antibiotic-fed groups was started earlier and was almost double that of the control group. The few cases of scours present occurred only among the calves in the control group. Results at 12 wk. of age will be presented.

P97. Utilization of urea by young dairy calves as affected by aureomycin. L. D. BROWN, D. R. JACOBSON, J. P. EVERETT, D. M. SEATH, AND J. W. RUST, University of Kentucky, Lexington.

In an 84-day experiment utilizing 48 two-day-old Holstein and Jersey calves, a calf starter made up of 0.3 ground, medium-quality timothy hay, corn, oats, molasses, corn starch, and urea, and containing 12.1% protein equivalent (46.3% of N supplied by urea), was found to be adequate for above-normal growth of dairy calves. Limited milk was fed to 42 days of age. No long hay was fed. Starters One through Four, containing 6.5, 9.4, 12.1 and 15.3% protein equivalent, led to average daily gains of 0.54, 1.02, 1.29, and 1.19 lb., respectively. Apparent digestibility of crude protein and dry matter, as well as nitrogen retention, were significantly lower for Group One than for the other groups. Nitrogen retention increased significantly with age. Blood urea and plasma protein was significantly higher at two days of age than at 4, 8, or 12 wk. of age. Blood urea for Group Four was significantly higher than for Groups One and Two. Feed consumption and efficiency values present trends very similar to average daily gain. Aureomycin fed to half of the calves significantly affected average daily gain, heart girth, and feed efficiency.

P98. Digestive enzyme activity in the young calf. J. T. HUBER, P. A. HARTMAN, N. L. JACOBSON, AND R. S. ALLEN, Iowa State College, Ames.

Twelve male calves were divided into two comparable groups at birth. One calf in each group was sacrificed at 1 day and at 1, 2, 3, 5, and 6 wk. Fourteen hours prior to sacrifice, calves in Group I received a test meal of whole milk plus 3% lactose, and Group II received whole milk plus 2.5% sucrose and 0.5% starch. Except for calves sacrificed at one day, diets similar to the respective test meals were fed from four days of age to sacrifice (colostrum, first three days). Gastric pepsin; pancreatic protease, lipase, and amylase, and intestinal lactase, maltase, and sucrase activities in the respective tissues were estimated. Test-meal residues in segments of the gastrointestinal tract were analyzed to determine nutrient digestion. Lactase decreased with age and, except for animals sacrificed at one day, was less in the posterior one-third of the small intestine. Maltase did not change with age or diet; no sucrase was detected. Pepsin was highest at 1 wk. and decreased thereafter. Lipase, pancreatic proteease, and amylase increased during the first week. Carbohydrate digestion was 91 and 76% for Groups I and II, respectively. Scouring was greater in Group II. Average pH of the contents of the large intestine was 6.80 for Group I and 5.56 for Group II.

P99. Effects of the duration of whole milk feeding on growth of dairy calves. R. A. ACKERMAN, R. L. STEELE, AND H. O. HENDERSON, West Virginia University, Morgantown.

Two groups of 26 dairy calves of various breeds were fed and managed the same, except for the duration of whole-milk feeding, to determine if differences could be detected in growth and digestive disorders. One group of calves received 399 lb. of whole milk per calf from birth until they were 8 wk. of age; the other group received the same amount of milk per calf, but it was fed until the calves were 12 wk. of age. Hay and a starter were fed, free choice, through 16 wk., then the concentrates were limited and changed to a simple grain mixture. The results reported are on pooled data from two trials (the second trial is still in progress). The data were analyzed by analysis of variance, using the method of fitting constants. The first year's results show that the group fed milk for 12 wk. average 30 lb. heavier than the group fed the milk for only 8 wk., which approaches statistical significance. The 12-wk. group also required somewhat less TDN per pound of gain. Both groups were above normal in growth and remained in excellent health.

P100. Value of pasture for young dairy calves. M. C. STILLIONS, C. H. NOLLER, AND B. W. CROWL, Purdue University, Lafayette, Indiana.

Twenty-two Holstein calves were assigned at random to three experimental groups: Group 1, control (barn-fed); Group 2, alfalfa-ladino-brome pasture, and Group 3, same as Group 2 plus hay free-choice. The calves were started on experiment as early as three days of age and on pasture between eight and 14 days of age. Limited whole milk was fed to 49 days of age. The calves consumed an average of 64.5, 43.4, and 36.9 lb. grain, and 44.5, 0.0, and 10.1 lb. of hay, respectively, during the first 60 days. The average daily gains were 0.97, 1.14, and 1.10 lb., respectively. The differences in average daily gains were not statistically significant. From 60 to 116 days of age the calves in the respective groups consumed an average of 187.3, 110.0, and 107.9 lb. grain and 256.2, 0.0, and 20.5 lb. hay, with an average daliy gain of 1.46, 1.67, and 1.70 lb. The differences in average daily gains were not statistically significant. Fecal egg counts for parasites were negative throughout the experiment. Observations suggest that the use of hay is instrumental in improving fecal firmness and the general appearance of young calves on pasture.

P101. Development of rumination in the young calf. E. W. SWANSON AND J. D. HARRIS, JR., Department of Dairying, University of Tennessee, Knoxville.

Observations of calf activity were made at 5-min. intervals for 24-hr. periods every 2 wk. until calves were 16 wk. old. A total of 16 Jerseys and 16 Holsteins was used. Four of the Jerseys observed and 13 other Jerseys were killed at from 2 to 3 wk. of age, to check the gastrointestinal contents. The calves were kept in individual pens. They were fed milk and high milk-content replacer at from 10 to 15% of body weight daily for 6 wk. Pelleted calf starter, chopped mixed hay, and water were available at all times after three days of age. At six to 14 days of age, 79% of the Holsteins and 64% of the Jerseys were ruminating. By 20 to 28 days, all of the Holsteins and 91% of the Jerseys were ruminating. During the 34- to 42-day observation period, the Holsteins ruminated 4.6 hr. and the Jerseys 5.0 hr. per day. In this same time (24 hr.), the number of separate rumination periods averaged 11.7 for Holsteins and 12.6 for Jerseys, with little subsequent increase until the 16 wk. of age. Examination of rumen contents showed that 11 calves killed at from 14 to 22 days had been ruminating normally, and six killed at from 12 to 15 days of age had not been ruminating effectively. These data indicate that effective rumination begins in the hand-fed calf before it is 3 wk. of age.

P102. Magnesium ammonium phosphate precipitated in rumen fluid and the relationship to frothy bloat. R. A. PHELPS, C. K. SMITH, AND C. F. HUFFMAN, Michigan State University, East Lansing.

Eight rumen-fistulated dairy cows, six of which were sets of identical-twins, were fed either two froth-producing or five nonfroth-producing rations. Rumen ingesta from these animals was centrifuged at an RCF of 30,000 and the supernatant adjusted to pH 9 with 10% NH_4OH. The precipitate obtained was qualitatively and quantitatively analyzed. Elemental, flame, and infra-red spectrographic analyses revealed mainly $MgNH_4PO_4 \cdot 6H_2O$ in the rumen fluid precipitate. The quantitative determinations revealed an average of five times more $MgNH_4PO_4$ from the froth-producing rations than from the nonfroth-producing rations. The quantity of $MgNH_4PO_4$ formed appeared to be directly proportional to the rumen-ingesta acidity. The amount precipitated was greatly stimulated by feeding small amounts of corn, but was not directly dependent on the amount of NH_4OH added, or on the apparent Mg and P intake of the animals. A correlated in vitro study of foam stability revealed a stable foam in rumen fluid below pH 4.5 and an unstable foam from 7.0. The entire study is being continued and additional data will be presented.

P103. Effect of a scabrous and a nonscabrous diet on rumen motility and intrarumen pressure. H. W. COLVIN, JR., S. D. MUSGRAVE, AND G. F. WILLIAMS. Oklahoma State University, Stillwater.

Four Ayrshire bulls were fed a nonscabrous diet consisting of a concentrate mix and a roughage in the form of a wood cellulose product, Solka-Floc BW-20, from 6 to 41 mo. of age. In 19 rumen motility trials, conducted when the bulls were 35 mo. old, it was found that the average fasting rumen contraction frequency per minute, primary contraction amplitude (cm. water), and intrarumen pressure (cm. water) were 2.1, 9.1, and 1.8, respectively. Not only were the bulls bloated during the motility trials but also before this, and up to the time scabrous material was included in the diet. When the bulls were 41 mo. old, the Solka-Floc BW-20 in the diet was replaced by prairie hay. Within 4 wk., the average fasting rumen contraction frequency per minute, primary contraction amplitude (cm. water), and intrarumen pressure (cm. water) were 1.6, 12.2, and −4.9, respectively. These values are within the normal range for cattle. The results suggest that scabrous material in the diet is necessary to maintain the tonic activity of the central pool of motor neurons responsible for rumen motility and, perhaps, eructation.

P104. Effects of constant environmental temperature (50 and 80° F.) on growth responses of Brown Swiss, Holstein, and Jersey calves. H. D. JOHNSON, C. S. CHENG, AND A. C. RAGSDALE, University of Missouri, Columbia.

Two groups of dairy calves (three each of the Brown Swiss, Holstein, and Jersey breeds) were subjected to constant environmental temperatures (50 and 80° F.) from 1 to 12 mo. of age. Body weight, heart-girth and wither-height measurements indicate that Holstein and Jersey calves generally did better at 50 than at 80° F.; whereas, Brown Swiss at 80° F. made better gains than at 50° F., as indicated by the following breed averages at 12 mo.: Body weight (Kg) Brown Swiss, 325.50; Holstein, 360.33; Jersey, 219.67 at 50° F.; and 334, 325.67, and 217.17, respectively, at 80° F. Conformation measurements suggest a greater ratio wither-height:chest-girth at 80 than at 50° F. in all breeds. Mature weight (Kg) predicted with the aid of modified exponential equation $W = A - Be^{kt}$ are Brown Swiss, 700, Holstein, 700, Jersey, 460 at 50° F.; corresponding values at 80° F. are 650, 660, and 440 kg. Percentage monthly declines in instantaneous growth at 50° F. are Brown Swiss, 5.9; Holstein, 6.2; Jersey, 5.7; and at 80° F., Brown Swiss, 6.3; Holstein, 5.8, and Jersey, 5.5.

P105. Effect of rising environmental temperatures (35–95° F.) on thyroid I^{131} activity of Holstein, Brown Swiss, and Jersey heifers.

H. D. JOHNSON, University of Missouri, Columbia.

Rising environmental temperatures depressed dramatically the in vivo thyroid I^{131} release rate of dairy heifers. Two groups of dairy calves (three of each breed—Holstein, Brown Swiss, and Jersey) weer raised from 1 to 12 mo. of age at constant 50 and 80° F. temperatures. Following the growth experiment, the heifers were exposed progressively (2- to 3-wk. periods) from 35 to 95° F. Relative humidity was maintained constantly at approximately 60%. Average K values of thyroid I^{131} activity at 35° F. for Holstein, Brown Swiss, and Jerseys raised at 50° F. were 0.10, 0.09, and 0.147, respectively, and 0.105, 0.139, and 0.171, respectively, for 80° F.-reared heifers. At 90° F., these values declined to 0.041, 0.049, and 0.084 for the 50° F. Holstein, Brown Swiss, and Jersey heifers, and to 0.053, 0.045, and 0.050, respectively, for the 80° F. heifers. Adaptation was evident in the above data, as the 80° F. heifers (those raised at 80° F.) showed a greater I^{131} thyroid activity at the lower temperatures (below 80° F.) than the animals raised at 50° F. (Supported by AEC.)

P106. Sex differences in certain bovine blood characters. A. B. SCHULTZE, M. PLUM, M. G. A. RUMMERY, University of Nebraska, Lincoln.

Erythrocyte, leucocyte, and eosinophil counts, as well as hematocrit, glucose, and hemoglobin values, of the blood of six Holstein heifer and six Holstein bull calves were determined at irregular intervals during the first year of life. There appeared to be no significant differences between the sexes in eosinophil or leucocyte counts. Erythrocyte count, hematocrit, and hemoglobin values were nonsignificantly higher in the blood of heifer calves up to about 6 mo. of age. After 6 mo. and up to 1 yr. of age these blood constituents were significantly higher in the bull calves. From 6 mo. to 1 yr. of life, the hemoglobin level of the males averaged 1.3 gm/100 ml. higher; the erythrocyte count, 1.7 million per $mm.^3$ higher, and the hematocrit 5.2% higher than the same constituents in heifers at corresponding ages. Glucose levels in male calves appeared to decline after birth less rapidly than they did in heifer calves, although the differences between sexes in rate of decline with age were not statistically significant.

P107. Studies of growth in Holstein, Ayrshire, Guernsey, and Jersey females as determined by: (1) Comparison of measurements at several ages to birth measurements as an index; (2) comparison of measurements of other breeds with Holsteins as an index. H. P. DAVIS AND I. L. HATHAWAY, University of Nebraska, Lincoln.

Data from 20 yr. of monthly body measurements of growth have been assembled, for three measurements each of heights, lengths, and widths and two each for depths and girths and also for weight. Breed comparisons for each measurement in terms of birth as an index are shown in charts for ages 1 through 5 yr. A composite of 13 linear measurements at 5 yr. showed the following increases in size: Holsteins, 2.57; Ayrshire, 2.53; Guernseys, 2.55, and Jerseys, 2.66 times birth measurements. The data show how animals increase body size during the first 5 yr. of life. Charts show the relative position in terms of Holsteins as a standard, for each of the other breeds, for weight and the 13 linear measurements for the first 5 yr. of growth. Each chart is a composite of 13 age measurement relationships from birth to 5 yr. Each type of linear measurement is also composited. Considering a composite of all 13 measurement relationships: Holsteins were 100; Ayrshires, 93.7; Guernseys, 92.0, and Jerseys, 88.1%.

P108. Heritability of gestation length in dairy cattle. J. C. DEFRIES, R. W. TOUCHBERRY, AND R. L. HAYS, University of Illinois, Urbana.

The objective of the present study was to determine what fraction of the variance of gestation period is genetic and, thus, to indicate the possibilities of shortening gestation length. From 2,063 gestation periods of the five dairy breeds in the University of Illinois herd, the within-breed, service-sire component of variance accounted for 10.5% of the total variance after adjusting for sex of the calf; thus, heritability was found to be 0.42. From the within-breed regression of offspring of dams involving 1,125 pairs, heritability was found to be 0.47. The difference of 0.05 between these two estimates could be caused by sampling errors or by the additive genetic effect of the dam on gestation length. The repeatability of gestation periods of the same cow was 0.20. These estimates of heritability indicate that the gestation length of dairy cattle may be shortened by selection, but experimentation is necessary to determine if any deleterious effects would be associated with this change.

P109. Relative merits of five ways of adjusting for the herd influence on artificially sired (AI) daughters' records. S. N. GAUNT, University of Massachusetts, Amherst.

Previous research at this station gave estimates of the intra-sire regression of the AI progeny record on each of the following contemporary averages: *(1)* The annual actual DHIA herd average, and stable-mates' average for *(2)* annual ME lactation, *(3)* 6-mo. ME season, *(4)* 7-mo. running ME season, and *(5)* annual actual age group. The average estimated regressions for milk and fat (ranging from 0.84 to 0.98) were used to adjust 4,590 and 1,404 AI progeny records of 296 and 145

Holstein and Guernsey sires for each contemporary average as: Daughter—b (herd or stablemate average—AI breed average). A variance component analysis (pooled data) of the adjusted records gave estimates of the herd component of 422, 123, 193, 111, and 308 for the respective measures (1, 2, 3, 4, and 5), as compared to 3.779 for *(6)* the adjusted record. Sire component estimates for *(6)* and *(5)* were highest, apparently due to inclusion of year effects, and in *(5)*, possibly, failure to remove fully sire-progeny age differences. Sire-herd component estimates were negligible. Further testing of these measures, using later proofs of these sires, is in process.

P110. Preliminary repeatability estimates of per cent total protein and other constituents in milk. S. D. MUSGRAVE, R. E. WALTON, F. A. GRAYBILL, R. MACVICAR, AND J. B. MICKLE, Oklahoma State University, Stillwater.

Data presented herein are based on 77 lactations from 63 Holstein cows. Daily composite samples of milk of individual cows were taken at monthly intervals. An average of 9.6 samples was obtained per lactation. Fourteen cows had two lactations. All lactations were initiated during the years 1953–1954. Repeatability estimates were computed by the usual least-squares procedure. When a single average value was used to represent each lactation, repeatability estimates were obtained as follows: *(a)* 0.2346 for per cent total protein (Kjeldahl method); *(b)* 0.0795 for per cent fat (Mojonnier method); *(c)* 0.5397 for per cent TS (Mojonnier method); *(d)* 0.4241 for per cent TS (Cenco procedure); *(e)* 0.6711 for per cent SNF difference from Mojonnier), and *(f)* 0.3227 for pounds of milk produced per sampling day. When individual monthly values were used to evaluate repeatability within lactations, then estimates were obtained as follows: *(a)* 0.1233 for per cent total protein; *(b)* 0.1261 for per cent fat; *(c)* 0.0967 for per cent TS (Mojonnier); *(d)* 0.1872 for per cent TS (Cenco); *(e)* 0.2188 for per cent SNF (difference from Mojonnier), and *(f)* 0.2360 for pounds of milk per sampling day.

P111. Error in estimation of lactational yields of solids-not-fat in milk from sampling one day a month. N. R. THOMPSON AND G. C. GRAF, Virginia Polytechnic Institute, Blacksburg.

Samples of individual milkings from 12 Holstein-Friesian cows were taken twice a day throughout the lactation period, and combined to make one-day composite samples. Percentage of butterfat was determiner by the Babcock method. Percentage of total solids was determined through use of the Watson lactometer and formula. Percentage of solids-not-fat was obtained by difference. Lactation yields of milk, butterfat, total solids, and solids-not-fat were obtained both as the sums of actual daily yields and as the sums of sampling-day yields, multiplied by days in testing periods. The high, low, and unweighted mean percentage deviations of calculated lactational yields from actual were: Milk, +1.2, −4.6, −2.3; butterfat, +4.1, −5.1, −1.6; total solids, +1.4, −4.1, −2.0, and solids-not-fat, +1.5, −4.8, −2.4.

P112. Genetic variation in clinical mastitis. C. W. YOUNG, J. E. LEGATES, AND J. G. LECCE, North Carolina State College, Raleigh.

The heritability of clinical mastitis was investigated where quantitative scores for clinical mastitis were determined on a lactation basis. Estimates were obtained from 416 complete lactations on 285 cows in four state-owned North Carolina herds. Relative efficiencies of selection based on indexes including laboratory findings, in addition to clinical mastitis and clinical mastitis alone, were compared. Also studied was the relationship of clinical mastitis to udder height. The heritability estimates obtained from the data were 0.71 from the paternal-sister correlation, and 0.21 from the daughter-dam regression. In addition, repeatability estimates of 0.35 and 0.40 were obtained. From these estimates, and additional information on components of hereditary variance, a value of 0.24 was arrived at as a reasonable value for the heritability of clinical mastitis. The inclusion of bacteriological data and leucocyte counts from monthly milk samples in the selection criteria resulted in only a 0–5% increase in efficiency of selection against clinical mastitis. The correlation between udder height and clinical mastitis was −0.27 ($P < .01$) when herd and breed differences were removed, and −0.14($P < .05$) when additional differences owing to age were removed.

P113. Heritability and repeatability of milk-yield records of Holstein-Friesian cows in Louisiana with and without certain environmental corrections. C. BRANTON, E. J. STONE, AND G. D. MILLER, Louisiana State University, Baton Rouge.

Considerable research has been done on the environmental corrections of milk-production records in the temperate regions of the world. However, very little has been done in the subtropical areas, such as the Gulf Coast region of this country. Therefore, such studies were initiated on the records in the L.S.U. Holstein-Friesian herd. Age-correction factors were derived by the multiplicative method, from data on 1,837 lactations of 406 cows during the period 1927–1956. It was found that the current breed factors underconvert the records of young cows by a considerable amount. Body weight was much more highly correlated with milk yield than was age at calving. Studies of the year and season-of-freshening effects on 722 lactations of 283 cows, during the period

1947–1957, revealed that both had highly significant effects on milk yield. At present, the heritability and repeatability estimates on these records from daughters of 31 bulls are being calculated on the records, before and after appropriate corrections for the effects of age at calving years and seasons of freshening. Daughter-dam comparisons on these bulls also are being considered.

P114. Estimates of environmental trends and biases resulting from errors in age factors and repeatability. C. R. HENDERSON, Cornell University, Ithaca, N. Y.

Unbiased evaluation of noncontemporaries from records requires estimation of intra-herd environmental trends and consequent adjustment of records. The author presented a method for doing this at the 1949 meeting of this Association. The required computations are laborious; consequently, it was not practical at that time to try the method on many herds. Recently, these computations have been programmed for the IBM 650, and a study has been made of 1950–1954 records in 11 New York herds. Using New York age factors and repeatability $=0.40$, the environmental change was estimated to have caused an average decline per year of 3.0 lb. fat per cow. Since the method requires age adjustments and a choice of repeatability, the effect of errors in these factors on the estimation of environmental trend was investigated. For each pound too much by which first-lactation records are built up, the environmental trend is biased downward by 0.22 lb. per cow per year. For each 0.01 by which the repeatability actually used in the estimation procedure exceeds true repeatability, the down bias is 0.08 lb. fat per cow per year.

P115. Analyses of feed efficiency on six sire groups in the Beltsville Holstein herd. N. W. HOOVEN, JR., AND C. A. MATTHEWS, Dairy Cattle Research Branch, Beltsville, Maryland.

One hundred and four cows by six sires were used in this study. Milk production and feed consumption data were compiled from the first lactation records on all cows. Second-lactation records were available and used on 66 cows, representing three of the six sire groups. Feed efficiency as used in this study was defined as:

$$\frac{\text{lb. of FCM}}{\text{lb. of TDN consumed}}$$

Analyses of variance indicated significant differences between sire groups with respect to feed efficiency. A high correlation was also found between feed efficiency and milk production. A covariance analysis was made, holding milk production constant. Results showed that significant differences remained between sire groups. This suggested that at least some of the genetic differences in feed efficiency are independent of milk yield. An intra-class correlation on 66 cows with first- and second-lactation records was made, to determine the repeatability of feed efficiency and FCM (ME). Results showed that the repeatability for feed efficiency was about two-thirds as high as for milk production.

P116. Relations between weight at first calving and milk production during the first lactation. R. H. MILLER AND L. D. MCGILLIARD, Michigan State University, East Lansing.

Michigan DHIA–IBM records for 4,677 Holsteins, 1,001 Guernseys, and 501 Jerseys were used to fit constants by least squares for the independent influences of age and weight on production in the first lactation. These effects indicated that delaying calving of heifers is economically disadvantageous, and that heavier heifers have little or no advantage over lighter heifers of similar age. Intraherd partial regressions were about 75 lb. of milk per month of age and 200 lb. of milk per 100 lb. weight at first calving. Large correlations between herd averages of weight and production contributed significantly to larger partial regressions of milk on weight, when herds were ignored. Genetic correlations between weight and production were of the order of 0.3. Heritabilities based on paternal-sib correlations ranged from 0.3 to 0.5 for weight and from 0.2 to 0.4 for production in the first lactation. Dairy heifers should be bred as soon as sufficiently large to minimize possible harmful effects on length of productive life. Selection for increased milk production may result in larger size. Correcting production for weight may remove some genetic variation in production.

P117. Relation between weight and production in dairy cattle. B. R. FARTHING AND J. E. LEGATES, North Carolina State College, Raleigh.

This study was initiated to determine the relative influence of live weight and age on production in Holstein and Jersey cows, and to develop a method of utilizing live weight as an aid to selection. The Holstein data consisted of 690 normal lactations and heart-girth measurements of 580 cows from ten North Carolina state institutional Holstein herds. One Jersey herd provided 396 normal lactations and weights of 175 cows. The weight measurements were taken the first month after calving. All lactations were expressed on a 305-day, 2× basis. The within-cow linear and quadratic effects of age and weight indicated that age is more effective than weight in accounting for variance in actual production. In the Jersey data, the intra-cow regression of mature equivalent (M.E.) production on live weight did not account for an appreciable portion of the variance in M.E. production. In the Holstein data, increases of 390 lb. in M.E. milk and 16 lb. in M.E. fat were associated with an average increase of 100

lb. in live weight. The greatest expected genetic improvement in M.E. milk was obtained when weight-corrected M.E. milk was used as the basis for selection.

P118. Relating age-conversion factors to herd level. S. R. SEARLE AND C. R. HENDERSON, Cornell University, Ithaca, New York.

In appraising animals on production records, those of young cows are usually adjusted by age-conversion factors. In no case have such factors been related to the level of the herd in which the cows make their records. This has been considered in a study of 20,432 cows in 749 Holstein, or predominantly Holstein, herds in New York State, in the four seasons, fall, 1950, through spring, 1952. For each herd-year-season, the cows younger than 5 yr. were divided into four groups, and regressions estimated of the difference between each age-group average and the 5- to 9.5-yr.-old average, on the herd average; they were found to be significantly different from zero. With these regressions, an age-corrected herd average can be calculated from the actual herd average, and the age-conversion factors for a herd are then based on the regression lines, using the age-corrected herd average. These regressions indicate that in high-producing herds the corrections for age should be greater than in low-producing herds. On the average, the resulting factors are close to the multiplicative ones now being used in New York State, suggesting that herd differences are largely, but not wholly, taken account of by such factors.

P119. Effect of season, age, and stage of lactation on type ratings of Holstein cows. C. J. WILCOX, K. O. PFAU, R. E. MATHER, AND J. W. BARTLETT, New Jersey Agricultural Experiment Station, Sussex.

Spring- and fall-type ratings by official inspectors from 1945 to 1957 were coded to maintain equal intervals (1.0) between ratings. Lactation number was used to represent age, with scores grouped into first lactation, second lactation, and third to fifth lactations (mature). Each lactation was divided into three periods, *(a)* parturition to 60 days, *(b)* 61 to 240 days, and *(c)* 241 days to next parturition. Only cows with five or more ratings were included in the study. Least-squares analyses, using dummy variables, yielded estimates of the various environmental effects independent of each other. Over-all ratings for fall were lower than spring by a coded value of 0.09 ($P<0.08$); first- and second-lactation scores were lower than mature scores by values of 0.52 and 0.29 ($P < 0.01$), respectively. Early and mid-lactation scores were similar, but late-lactation scores were 0.15 lower ($P < 0.01$). Subratings for fall, and for first and second lactations, were generally lower than their counterparts. Scores in late lactation were higher, except for dairy character, with scores of 0.19 lower ($P < 0.01$) than mid-lactation scores. These results indicated that adjustments for the significant influences would result in more accurate studies in this field.

P120. Association of certain type evaluations and type scores of first-lactation Holstein females. E. L. AKINS, T. M. LUDWICK, H. E. RICKARD, E. R. RADER, AND W. M. ETGEN, The Ohio Agricultural Experiment Station, Wooster.

Two hundred and seventy-three Holstein females and their 22 sires and dams (seven herds) were evaluated for type on a detailed score-card basis, to determine the relative influence of various type information in predicting the type of the daughter at the time of her first calf. Evaluations were made on the females at three early ages (3, 6, and 12 mo.), and 3 mo. after first-calving. Sires were evaluated only at maturity (5 yr.). Interpretation of the analyses indicates the following associations: *(a)* With the exception of the head-and-neck category, the breakdown classification of the sire had little value in predicting the type of his daughters; *(b)* no significant predictions for daughters' type could be made from information on the dam; *(c)* estimates of type-score repeatabilities ranged from 0.2 to 0.5 among younger ages (3 to 6, 3 to 12, 6 to 12 mo.); *(d)* repeatability of type scores from younger ages to after-calving was very low, *(e)* in general, all udder components had low repeatabilities and predictabilities.

P121. Heritability of various characteristics of body conformation and their association with production of Holstein-Friesian cows in Canada. D. G. GRIEVE, J. C. RENNIE, AND G. E. RAITHBY, Ontario Agricultural College, Guelph.

The official production and type classification records of 17,818 Holstein-Friesian cows distributed in 2,192 herds were used in this study. All records were completed during a 2-yr. period, 1953 to 1955. The production records were standardized, using two systems; namely, mature equivalent and breed class average. The intra-herd correlations between paternal half-sibs were used in estimating the heritability of the 18 different type characteristics listed under the sections on "Rump" and "Legs and Feet" on the official Holstein-Friesian Selective Registration score-card. These characteristics and the heritability estimates are as follows: High pelvis, 0.17; narrow rump, 0.11; low thurls, 0.14; low pins, 0.10; high tail-head, 0.10; coarse tail-head, 0.12; legs too straight, 0.09; sickled legs, 0.15; coarse legs, 0.16; close hocks, 0.13; thick thighs, 0.00; weak pasterns, 0.24; toes out (front), 0.15; toes out (rear), 0.13; shallow heel (front), 0.25; shallow heel (rear), 0.15; open-toed (front), 0.00, and open-toed (rear), 0.21. Phen-

otypic correlations and regressions between eight of the type characteristics and milk and butterfat production, calculated on an intra-herd and sire basis, were all small in magnitude. The correlations between these eight type characteristics and the percentage of butterfat were in all cases essentially zero.

P122. Contribution of breed of sire, breed of dam, and system of mating to rate of growth in crossbred as compared with purebred dairy cattle. D. C. SHREFFLER AND R. W. TOUCHBERRY, University of Illinois, Urbana.

Forty-three purebred Holsteins and Guernseys and 44 reciprocal Holstein-Guernsey crosses were compared, with respect to body weight, wither height, chest depth, heart girth, paunch girth, and lengths from withers to hips, hips to pinbones, withers to pinbones, and shoulder to pinbones. Measurements at 3, 6, 12, 18, 24, 30, 36, and 48 mo. of age were analyzed by the method of least squares; the normal equations were solved by using an inverse matrix. The effect of breed of dam was highly significant for all measurements at all ages, and in every instance was larger than the effect either of breed of sire or of mating system. The effect of breed of sire became significant in general at about 12 mo., but was not significant for the lengths from hips, withers, or shoulder to pinbones. The effect of mating system was not significant, but absence of non-additive genetic effects associated with cross-breeding is not unexpected, when the relatively high heritability of thees measurements is considered.

P123. Effect of two systems of mating on butterfat production of six lines of Holstein-Friesian cattle. C. E. KRAEMER, W. J. TYLER, AND A. B. CHAPMAN, University of Wisconsin, Madison.

The data for this study were the first lactation butterfat records of 78 outbred ($F_x=0$) and 69 inbred ($F_x=0.13$ to 0.29) progeny in six sire lines of purebred Holstein-Friesian cattle at the Emmons Blaine, Jr. Experimental Farm, Lake Mills, Wisconsin. The lactations were begun over a 6-yr. period. After adjustment to a 2× M.E., 305-day basis, the data were fitted to a least-squares model, and constants were computed for the effects of year, line, and system of mating. A preliminary analysis showed that the interaction between years and lines within systems of mating was not significant. The analysis of variance showed that the differences among years and the interaction between lines and systems of mating were highly significant. The regressions of butterfat production on percentage of inbreeding for the six lines were −2.4, −2.4, −2.4, −2.7, −3.1, and −7.0 lb. Five of the six lines showed similar effects of inbreeding and it is apparent that one line contributed mainly to the interaction of lines and systems.

P124. Performance of artificially sired daughters and their contemporary naturally sired herd-mates in stratified levels of herd production. N. J. GUDERYON, E. L. CORLEY, AND J. W. DUCKWALL, University of Wisconsin, Madison.

The 2×, 305-day, M.E. production of artificially sired (AI) daughteers was compared with their contemporary naturally sired (NS) herd-mates on a within-herd, within-calving year basis. A total of 51,023 Holstein-Friesian and 13,725 Guernsey milk and butterfat records were studied, after adjustment for an unequal number of AI and NS lactations within herds and years. When all available records were used, AI-NS differences were +157 lb. milk and +9 lb. butterfat for Holsteins and −20 lb. milk and +2 lb. butterfat for Guernseys. When 11,945 first records were used, these differences were +163 ($P > .01$) and +8 ($P < .01$) for Holsteins and +89 ($P > .05$) and +4 ($P > .05$) for Guernseys. Herds represented by first records were stratified into low, medium, and high levels, according to actual yearly herd averages for butterfat yield. Holstein AI-NS differences were +10 ($P < .01$), +13 ($P < .01$), and +1 lb. of fat, and +225 ($P < .01$), +175 ($P < .05 > .01$), and +79 ($P > .05$) lb. of milk in low, medium, and high herds, respectively. Corresponding differences for Guernseys were 0, +7, and +5 lb. of fat and +79, +220, and −39 lb. of milk, none of which was significant. These data suggest that Holstein AI daughters are genetically superior to their NS herd-mates in low and average DHIA herds in Wisconsin.

P125. A method of determining adequate sample size to estimate heritability or repeatability from the analysis of variance. STANLEY WEARDEN, Kansas Agricultural Experiment Station, Manhattan.

Heritability *(h)* and repeatability *(r)* are often estimated by functions of an intra-class correlation obtained through the analysis of variance. In the completely random design under Model II, two variances are estimated: That among genetic groups (σ_g^2) and that within groups (σ_w^2). In repeatability, a genetic group consists of multiple records on the same individual. The ratio $\sigma_g^2/\sigma_w^2=\theta$ can be expressed as $P/(\frac{1}{a}-P)$, where P is the genetic parameter (h or r) and a is the coefficient of relationship among the individuals within a genetic group. An adequate sample is one containing a sufficient number of observations *(n)* per genetic group, to detect, with known probability, significant differences among groups when they exist. This concerns the power of the test $[\beta(\theta)]$, which is distributed according to the central F-distribution. By fixing the power, and guessing the minimum size of P, it is possible to solve the equation $n=\frac{1}{a}[F\beta/F\alpha)-1]$. $F\beta$ is the F-value corresponding to the power,

and F_a to the probability of a Type I error. The solution is iterative, because the sizes of the two F-values depend on their degrees of freedom, which are dependent on n.

P126. Genetic structure of dairy cattle herds in terms of additive and dominance relationships. A. E. FREEMAN AND C. R. HENDERSON, Cornell University, Ithaca, New York.

Data from 11 New York grade and registered Holstein herds were used to characterize the additive and dominance relationships within these herds. All possible additive relationships (a_{ij}) and the dominance relationship (d_{ij}) among non-inbred animals were computed, after tracing ancestry two generations beyond the foundation animals of each herd. Excepting ancestors of the foundation cows, the mean a_{ij} for all herds was 0.04. Distribution of these values showed that 73% of these animals were unrelated and 92% of the a_{ij}'s were less than 0.175. The mean coefficient of inbreeding was 0.007. The relationships among all non-inbred animals with a_{ij}'s > 0, whose complete records started between the ages of 22–34 mo., inclusive, were computed. The mean additive and dominance relationships were 0.13 and 0.007, respectively. For these animals, 77% of the a_{ij}'s were < 0.2 and 50% were < 0.1; also, 91% of the d_{ij}'s were < 0.025. These low relationships indicate that, regardless of the magnitude of the population dominance variance and the variance due to inter-allelic interactions involving both genes of at least one pair, the contribution of these components could, in the average, contribute very little to the total genetic covariance between relatives in a random-mating population.

EXTENSION SECTION

E1. A device to pipette samples directly into test bottles. W. R. DAVEY, The Pennsylvania State University, University Park.

Two studies were made, using procedures which should reduce the time and work involved in conventional D.H.I.A. sampling. The first study consisted of removing 8.8 ml. of milk from the thoroughly mixed milk, with a properly calibrated pipette, after each milking, and introducing it into a milk test bottle. The second study made use of the Cornwall Leur-Lok syringe with appropriate attachments. After adjusting delivery to 8.75 ml. of water, samples of nights' and mornings' milk were delivered to the test bottle, similarly to the method used in the first study. The data revealed that in 853 comparisons with the 8.8–ml. pipette, 86.6% of the tests fell within 0.1% of the control procedure and 97.6% checked within 0.2%. In the method using the syringe, 81.3% of the results fell within 0.1% of the control procedure and 96.3% checked within 0.2%, based on 621 comparative tests. The method would seem to provide an improvement over present procedures, resulting in less sample handling and les schance of erroneous results owing to souring or churning. However, this procedure would preclude aliquot sampling when the interval between milking caused extreme variations in milk weights.

E2. Results of accuracy studies on weighing and sampling devices. M. E. SENGER, North Carolina State College, Raleigh.

Continued expansion in production testing will require simple and convenient methods for weighing and sampling milk in pipeline systems. At the American Dairy Science Association meetings in June, 1957, weighing and sampling devices for use in the National Cooperative Dairy Herd Improvement Program were approved. Accuracy tolerances were suggested as a guide in determining which devices should be acceptable for standard DHIA work. With the increased popularity of pipeline milkers, many devices for weighing and sampling milk from individual cows are now available. However, some of these devices have not been adequately checked and tested for accuracy under proper and uniform conditions. Consequently, those supervising DHIA testing have not had the information needed to determine which devices are within the acceptable accuracy tolerances. A survey was made of extension dairymen supervising DHIA testing, to find out which devices have been adequately tested and checked for accuracy. A summary of this survey, and reports by several states on the results of acuracy studies on weighing and sampling devices, will be given.

E3. Motivation of supervisors and dairymen in identifying cows in Dairy Herd Improvement associations. L. H. RICH AND B. H. CRANDALL, Utah State Agricultural College, Logan.

The incentive for identifying large percentages of D.H.I. cow records is of interest, and prospective results indicate that this can be done with minimum effort. Mechanical processing gives legibility, accuracy, and completeness in reports, provided supervisor's monthly barn sheets arrive at computing service listing correct data. The supervisor's job then is largely completed. Cows once identified with acceptable requirements for sire provings carry such identified listings permanently, with changes in status, lactation data, etc. as accumulative periods occur. Supervisors (full- or part-time), carefully selected and rewarded for time expended; D.H.I. officers given responsibility; dairymen kept fully informed; national eartagging program with cooperating agencies, and computing service personnel showing affability, accuracy, dispatch, and informative attitudes are factors making for herd permanency and for obtaining more complete identifications. From 79 to 81% of 305-day records processed monthly were acceptable in 1957 for the National Sire Proving Program (sent in card-lots). Covering a 3-yr. period, data were processed,

providing listings for 1,323 sires and their respective daughters' records (by sire). Processing of mature equivalents monthly, for properly identified breeding records only, gives incentive for cooperation of dairymen and supervisors. Working materials, district schools, and close relationship of dairymen, supervisors, computing service and extension personnel, are paramount. Success is its own reward.

E4. Comparison of machine-calculated records and lactations submitted by D.H.I.A. fieldmen. A. G. SENDELBACH AND E. L. CORLEY, University of Wisconsin, Madison.

These data were taken from 43 standard D.H.I.A. Wisconsin herds in ten associations with 20 field supervisors. The 305-day lactation reports submitted routinely by supervisors were computed later with punch-card equipment. This provided an opportunity *(1)* to study the fieldmen's accuracy in computting records, and *(2)* to determine if production differences occur in reported and nonreported lactations. Over a 3-yr. period, 3,476 lactations were computed by machine, of which 2,926 (84%) were reported by fieldmen. Of these, 427 (15%) were in error as to calving date or cow identification. The remaining 2,499 were compared with the machine-computed records, regarding the frequency and percentage of error in milk and butterfat yield. Milk-fed errors in excess of 500 lb. totaled 162 (7%) of the reported records, whereas 134 (5%) were in error by 20 or more lb. of fat. The mean absolute error in milk yield was 117 lb. for all reported lactations and 575 lb. for the 508 records exceeding 1% in error. Reported records exceeded those not reported by 1,147 lb. milk, 60 lb. fat, and 32 days. After removing all records of less than 180 days, these differences were 730, 34, and 15.

E5. Comparison of age-conversion factors for Ayrshire butterfat records. R. D. CASSELL, R. S. DUNBAR, JR., R. G. MITCHELL, AND I. D. PORTERFIELD, West Virginia University, Morgantown.

Eighty thousand, four hundred and fifty-two Ayrshire butterfat production records, completed between January 1, 1946, and December 31, 1955, were used to develop additive and multiplicative age-conversion factors. The accuracy of these faetors was checked by applying them to immature butterfat production records on cows that freshened at from 72 to 95 mo. of age during the period between January 1, 1956, and May 31, 1957.

Intra-herd variances, repeatabilities, and correlations were used in comparing the present Ayrshire factors, the additive, and the multiplicative correction factors for predicting the animal's production at maturity. The variation among herds and among cows within herds was highly significant. The intra-herd repeatability value for the present Ayrshire factors was 0.474; the additive factors, 0.322, and the multiplicative factors, 0.471. Intra-herd correlations between adjusted immature and actual mature records for the three conversion factors were all significant at 2 and 3 yr. of age and highly significant at 4 and 5 yr. of age.

E6. Increasing efficiency on farms producing milk for manufacturing purposes. G. S. PARSONS, North Carolina State College, Raleigh.

In the last few years, many manufacturing milk producers with small investments have made their jobs easier, more pleasant, and more profitable. This has been done through the use of labor-saving devices, such as self-feeding of silage from horizontal silos, loose housing of cattle, ground-level hay storage, elevated-stall milking parlors, and the cow-to-can milking machine. Among the greatest labor-saving devices in the production of manufacturing milk have been the elevated stalls and the cow-to-can milker. The elevated-stall milking parlors are a simple arrangement in which the cows are elevated to allow milking without stooping. All milking operations, grain feeding, and the moving of cows in and out are done by one person, with little walking. Labor required for milking can be reduced one half. One person can easily milk 12–15 cows per hour. No elaborate building is needed. Almost every farm has a barn or other similar building in which a parlor can be installed. Through the use of these labor-saving devices, part of the family can work in industry and still maintain the dairy operation. Also, many physically handicapped people find that these arrangements enable them to continue milking when otherwise they could not.

E7. Managing large herds of 100 cows or more. W. R. VANSANT, University of Arizona, Tucson.

In 1952, it was observed that changes in herd management were taking place in Arizona. A yearly survey was conducted by DHIA supervisors and the following changes were revealed: increase in herd size, 30%; additional hired milkers, 19%; bulk-storage tanks, 58%; pipeline milkers, 34%; and decrease in raising replacements, 57%; raising hay, 25%; pasture, 27%. As a result of this study, an extension program was developed which included barn-layout designs, a herd-replacement program, artificial insemination, handling and treatment of individual animals, maternity quarters, feed storage, and many other recommendations to improve efficiency in herds of from 180 to 240 cows. More mechanization is required for large herds, such as crop-harvesting equipment, mechanical feeding wagons and trucks for soiling crops and for silage, special arrangements for feeding hay, use of mechanized devices for cleaning corrals, and many others to improve efficiency and save labor.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL HEALTH

189. Distribution of micrococci and other bacteria in milk samples from a single dairy herd after 12 yr. of mastitis control. O. W. SCHALM AND J. LASMANIS, Univ. of Calif., Davis. Am. J. Vet. Research, **18**, 69: 778. 1957.

Milk samples for bacteriological studies were drawn from a 250-cow herd over a period of 2 yr., which were the 13th and 14th yr. of a mastitis control program. Samples were tested by the Hotis test, growth on blood agar, coagulase test, and the Whiteside test. A total of 17,927 samples were processed.

The mean distribution of organisms was 70.8% micrococci, 11.2% coliforms, 8.5% *streptococci*, 1.0% *Proteus* species, 0.2% *Corynebacterium pyogenes*, 0.1% *Pseudomonas aeruginosa*, and 8.2% of all other organisms.

The percentage of micrococci that were pathogenic increased from 25.4 in the 1st lactation to 46.8 in the 5th or higher. It is postulated that if control measures had not been directed against the pathogenic micrococci they might have increased to higher percentages. *Streptococcus agalactiae* infection was eliminated from the herd easily at the start of the testing, and was again eliminated after its reappearance 10 yr. later. *Micrococcus pyogenes* infections were not completely eliminated by control and treatment procedures. At times, coliform organisms caused serious acute mastitis in the herd, but many of the positive coliform samples are considered to be contaminated. The Whiteside test was positive in 14.5% of the samples in which no organisms were found. It was positive in only 53% of the *S. agalactiae* and in 53% of the coagulase-positive micrococci infected samples. Its value for diagnosis of infection in treated herds is, thus, very limited.

E. W. Swanson

190. Diagnosis and treatment of mastitis. W. N. PLASTRIDGE AND H. H. HALE. Storrs Agr. Expt. Sta., Prog. Rpt. 14. 1956.

The control of *Streptococcus agalactiae* mastitis is possible when frequent laboratory testing of milk samples and the treatment of infected udders are carried on. Elimination of the organism from herds increased production an average of 10% in 91 herds. The rate of infection caused by other organisms can be reduced by the treatment of quarters found infected by tests made about every 3 mo.

A. W. Rudnick, Jr.

191. Distribution of aqueous penicillin and penicillin in oil in normal goat udders following intramammary injection—An autoradiographic study. S. ULLBERG, E. HANSSON, AND H. FUNKE, Royal Vet. Coll., Stockholm, Sweden. Am. J. Vet. Research, **19**, 70: 135. 1958.

The penetration of penicillin labeled with S^{35} into normal goat udders was shown by autoradiographic techniques. Photographs of the udder slices accompany the autoradiographs. By removing one-half of each udder at 1 and 8 hr. after treatment, the changes at 1 and 8 hr. were shown. Uniform distribution of the penicillin throughout the glands was shown as early as 1 hr. after using either oil or aqueous vehicles. The penicillin from aqueous solution penetrated the parenchyma to a somewhat greater degree than did that from oil.

E. W. Swanson

192. Distribution of penicillin in mastitic udders following intramammary injection—An autoradiographic study. S. ULLBERG, E. HANSSON, AND H. FUNKE, Royal Vet. Coll., Stockholm, Sweden. Am. J. Vet. Research, **19**, 70: 84. 1958.

Penicillin, labeled with S^{35}, was injected in oil or aqueous solution through the teat canal into six udders of cows or goats which had clinical mastitis. Fixed and frozen sections were photographed and also used to make autoradiographs to show the extent of penetration of the radioactive penicillin. Failure of the penicillin to penetrate some parts of the diseased udders was noted in every case. In the most severe inflammations, the penicillin was found only in the largest collecting ducts and cisterns. In less severe inflammations penicillin was distributed more uniformly. The use of oil or aqueous vehicles did not affect the penetration of the penicillin into diseased udders. It is

postulated that the failure of penicillin treatment of mastitis often results from the inability of the drug to reach the infected areas.

E. W. Swanson

193. Effectiveness of various measures in bloat therapy and prophylaxis and the resulting influence on rumen fluid characteristics. J. T. Blake, N. L. Jacobson, and R. S. Allen, Iowa Agr. Expt. Sta., Ames. Am. J. Vet. Research, **18**, 69: 756. 1957.

The effectiveness of a detergent (alkylaryl sodium sulfonate type) and trisodium phosphate in preventing bloat of cattle pasturing alfalfa was tested. Trisodium phosphate had little effect either on bloat incidence or on rumen fluid characteristics. The detergent at 20 g. per 1,000-lb. cow was only slightly effective, but at 30 g. it reduced bloat incidence and severity significantly. Rumen fluid from bloated animals was slightly lower than normal in pH and specific gravity, and slightly higher in viscosity and surface tension. Marked increases occurred in the degree of foaminess and in foam stability. A test of the effectiveness of detergent and cholesterol on bloat induced by alfalfa juice given by stomach tube gave essentially negative results. This indicates that the modes of action or types of bloat in these two instances are different. E. W. Swanson

194. A comparison of the effect of catalase-positive and catalase-negative vibrio on the fertility of cattle. A. H. Frank, J. H. Bryner, and B. Caruthers, ARS, USDA, Beltsville, Md. Am. J. Vet. Research, **19**, 70: 108. 1958.

Vibrio infection of bulls and heifers or cows, determined by bacteriological examination of preputial or vaginal mucus, showed that both catalase-positive (C+) and -negative (C−) vibrio infections occurred. Persistent C− vibrio were found in bulls that had never served a female. In one herd of 65 virgin heifers and 72 cows, six bulls in service were all infected with C− vibrio. The females spontaneously recover from the C− vibrio infection, so that all are free of it at first service. Following service, 15 of the 137 became infected. The herd averaged 1.6 services per conception. Another group of 27 virgin heifers and 91 cows were bred to C− vibrio-infected bulls in comparison with vibrio-free bulls. There was no significant difference in conception rate between the two groups of bulls. Only two of six heifers bred to C− vibrio-infected bulls shed the organism before pregnancy was established; whereas, all of six heifers bred to a C+ bull were easily infected, resulting in delays in conception. Catalase-positive vibrio were not found in virgin bulls or heifers. They were easily transmitted after infection occurred and produced prolonged infertility similar to that due to *Vibrio fetus*. One bull artificially infected with C+ vibrio eliminated that infection spontaneously, but remained infected with C− vibrio. No gross pathological changes or clinical signs were associated with vibriosis of bulls.

E. W. Swanson

BOOK REVIEW

195. Chemistry and Biology of Yeasts. Ed. A. H. Cook. Academic Press, New York. $22. 1958.

This comprehensive treatise on yeast includes the following contributions: The Classification of Yeasts by Lodder, Slooff, and Kreger-van Rij; Ecology of Yeasts by Lund; Life History and Cytology of Yeasts by Winge and Roberts; Aspects of the Chemical Composition of Yeast by Eddy; Yeast Growth by Morris; Fermentation and Respiration by Nord and Weiss; Synthesis and Degradation of Cellular Carbohydrates by Yeasts by Trevelyan; Nitrogen Metabolism by Harris; Technology of Yeast by Pyke; Pathogenic Yeasts by Ainsworth; Yeast in Food Spoilage by Ingram, and Flocculation of Yeast by Jansen. In addition, there are subject and author indexes.

The stated purpose of this volume is to bring together up-to-date, authoritative, and complete this aim has been admirably achieved. The aid technology of yeasts. Such a compilation is necessitated by the recent rapid expansion of fundamental research on yeasts, and the need to distribute a large amount of knowledge which hitherto has been unavailable in convenient form. It is the reviewer's opinion that this aim has been admirably achieved. The aid of outstanding authorities in the individual areas has been enlisted. The result is an assemblage of chapters, each of which is a monograph in itself.

The chapter on Fermentation and Respiration, for instance, presents details of the exciting epoch in enzyme biochemistry wherein the mechanism of glucose fermentation to ethanol was pieced together and the now classical Embden-Meyerhof pathway of glycolysis was established. Also included is an equally complete account of the complex reactions of glucose oxidation. This chapter will be of greatest value as a textbook to students of intermediary metabolism and to researchers as a ready reference. Similarly, the chapter on nitrogen metabolism is complete and meticulous in detail. In addition to a description of pathways of amino acid degradation and biosynthesis, a timely account of the status of adaptive enzyme formation and the mechanism of enzyme formation in yeast is included. As an example of the completeness of annotation, almost 460 references are cited in this chapter.

In a similar fashion, the chapter on classification, genetics, life history, and cytology are valuable because they consolidate the trends and current thinking. The sketches, photographs, tables, and charts are particularly effective in transmitting the subject matter of these areas.

It is satisfying to the reviewer that the discussion of the technological and practical aspects of yeasts are treated against the background of, or in proximity with, the fundamental chemistry and biology of the organism. The chapters on technology, flocculation, food spoilage, and pathogenic yeasts attest to the great economic importance of yeasts and provide the balance in perspective which is a major attribute of this work.

This volume will be a great teacher to the students of yeasts and an indispensable source of information both in university and industrial laboratories. W. A. Wood

196. Methods of Biochemical Analysis. Vol. 6. Ed., DAVID GLICK. Interscience Publishers, New York. 358 pages. $8.50. 1958.

The sixth volume expands again the usefulness of this series to the biochemist. Included in this volume are: New Developments in the Chemical Determination of Nucleic Acids, by J. M. Webb and H. B. Levy; The Microbiological Assay of Nucleic Acids and Their Derivatives, by H. K. Miller; The Determination of Formaldehyde and Serine in Biological Systems, by W. R. Frisell and C. G. Mackenzie; New Methods for Purification and Separation of Purines, by Felix Bergmann and Shabtay Dikstein; Assay of Serotonin and Related Metabolites, Enzymes, and Drugs, by Sidney Udenfriend, Herbert Weissbach, and B. B. Brodie; Determination of Transaminase, by A. J. Aspen and Alton Meister; Glycolipide Determination, by N. S. Radin; Methods for the Determination of Thiamine, by Olaf Mickelsen and R. S. Yamamoto; Rapid Electrophoresis in Density Gradients Combined with pH and/or Conductivity Gradients, by Alexander Kolin; and Determination of Hexosamines, by Sven Gardell. These articles are followed by author and subject indexes and the useful cumulative author and subject indexes to all six volumes.

The thoroughness with which most of the authors have treated their subjects makes this volume fully as useful as previous volumes in the series. The discussions and detailed procedures given provide both a comprehensive review of value to the biochemist acquainted with the field as well as to scientists in allied fields in need of a specific method for practical use. B. L. Larson

BUTTER

197. Individually wrapped butter servings. Anon. Milk Prod. J., **49**, 3: 10. 1958.

This process uses a machine which makes possible the combination of heat and refrigeration in the sealing of individual butter patties in cellophane, at a production rate of from 600 to 1,000 lb. of packaged patties per hour.

Advantages claimed for individually wrapped butter patties include the sanitation aspects of individual servings of butter, the elimination of man-hours required to slice or score commerical sheets of butter, and the reuse of butter patties not consumed in the institutional market. This procedure has yielded an over-all saving of 25% on butter costs in Army messhalls. J. J. Janzen

198. Whey cream butter. L. C. THOMSEN. Wis. Agr. Expt. Sta., Research Bull. 197. 1956.

Precautions for handling, heat treating, and churning whey cream are given.

A. W. Rudnick, Jr.

CHEESE

199. Manufacture of Cheddar cheese from pasteurized homogenized milk. I. I. PETERS, C. A. MULAY, AND G. L. DALTON, Dept. of Dairy Sci., Texas A & M Coll., College Station. Milk Prod. J., **49**, 3: 13. 1958.

Pasteurized milk homogenized at 500 or 1,000 p.s.i. yields cheese equal in flavor and superior in body and texture to that of pasteurized, nonhomogenized milk cheese.

Advantages other than those obtained by the use of pasteurized, nonhomogenized milk include *(1)* higher yield by more complete utilization of fat; *(2)* less trouble with fat leakage; *(3)* possibly lower shrinkage losses during ripening and storage of cheese; *(4)* absence of creaming during setting of milk, and *(5)* no churning during agitation of milk.

J. J. Janzen

200. Development of antibiotic resistance in cheese starter cultures. K. M. SHAHANI AND W. J. HARPER, Dept. of Dairy Technol., Ohio State Univ., Columbus. Milk Prod. J., **49**, 3: 15. 1958.

Nineteen cheese cultures differed widely in their sensitivity to penicillin and aureomycin. In general, the cultures tolerated higher concentrations of aureomycin than of penicillin. Four lactobacillus cultures which had been subcultured continuously in flasks, and were thereby adapted to a high S:D ratio for growth, tolerated much higher concentrations of antibiotics without loss of acid-producing ability than the parent cultures subcultured in test tubes.

Resistance to 10 p.p.m. of aeromycin was developed in these four lactobacilli. The induced resistant strains tolerated higher levels of aureomycin and terramycin but lower levels of streptomycin than their normal counterparts or naturally resistant strains. However, upon the development of resistance, the acid-producing ability was retarded from 6 to 20%. The induced resistant organisms produced adequate amounts of acid for use in the manufacture of some cheese varieties from milk containing up to 10 p.p.m. of aeromycin. These particular cultures would be limited to use where high-

temperature lactic cultures are desired, as in Swiss and Italian cheeses. J. J. Janzen

CONDENSED AND DRIED MILKS; BY-PRODUCTS

201. Pudding compositions and method of making a pudding. M. P. Castagna, D. S. Thomas, A. D. Campbell, and R. J. Carbonell. U. S. Patent 2,829,978. 18 claims. April 8, 1958. Offic. Gaz. U. S. Pat. Office, **729**, 2: 399. 1958.

This patent concerns sweetened dry pudding mix containing an edible pyrophosphate, an orthophosphate, and a calcium salt in amounts sufficient to gel the milk in which the powder is dissolved. Curdling of the milk is prevented by the presence of an edible thickening agent. R. Whitaker

DAIRY CHEMISTRY

202. Inhomogeneity of α-casein from goat's milk. T. Hofman. Nature, **181**, 4609: 633. 1958.

There are at least two electrophoretically distinct fractions of α-casein in freshly prepared casein from goat's milk. These fractions have the same P and N content as casein from cow's milk, but differ electrophoretically. Electrophoretic patterns are given. R. Whitaker

203. Studies on amino-carbonyl reaction of milk products. XVIII. On the presence of the secondarily formed ketose in evaporated milk. Susumu Adachi, Tohoku Univ., Sendai, Japan. Tohoku J. Agr. Research, **8**: 79. 1957. (In English.)

Studies were made on the occurrence of the sugar, lactulose, in commercial evaporated milk. This sugar was readily detected in four different brands involving 12 lots of evaporated milk. A quantitative method is presented for measuring lactulose content and in the analysis of 12 samples a range of from 365 to 940 mg. per 100 g. of product was found. Mechanisms of formation for this sugar are presented and it is pointed out that the presence of this sugar in evaporated milk may be a consideration of importance in its nutritive value and physical and chemical properties. S. Patton

204. Odor and flavor of methional. S. Patton and Isabel J. Barnes, Dept. of Dairy Sci., Penn. State Univ., University Park. Food Research, **23**, 2: 221. 1958.

These workers have been unable to verify the contention of Witting and Batzer (*Food Research, 22*: 237. 1957.) that pure methional is odorless and only acquires odor by exposure to air after preparation, which is assumed to be an oxidative change probably resulting in methional sulfoxide. On the contrary, the authors found freshly prepared methional to have a strong odor. Neither was any support obtained for Witting and Batzer's inference that in the series sulfide, sulfoxide, sulfone, the sulfoxide is most odorous, inasmuch as in such a series the sulfide was judged by far the most odorous. F. J. Doan

205. Irish moss. L. H. Berndt and R. A. Klein. U. S. Patent 2,830,903. April 15, 1958. 4 Claims. Offic. Gaz. U. S. Patent Office, **729**, 3: 647. 1958.

A process is described for treating Irish moss to produce a stabilizer for making a chocolate syrup which can be added to cold milk to make a nonsettling chocolate milk. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

206. The milk production—consumption balance and use of reserve supplies in the Connecticut market, 1954. N. Kogan, G. K. Brinegar, and S. Johnson, Storrs Agr. Expt. Sta., *Bull.* 324. 1955.

In a study of records of a sample of 12 milk handlers in Connecticut, it was determined that reserve supplies of fluid milk had been stabilized at from 10 to 12% in the 7-yr. period of 1949–55. Large handlers distributed about 31% of all milk, but 63% of the total surplus; medium handlers, 8% of receipts but 4% of surplus, whereas small handlers handled 0.5% of receipts and 0.05% of surplus. The small handlers, especially, with personal contact with producers, restrict production to correspond closely with their Class 1 sales. All surplus butterfat is used in cream and none is used for butter or for other manufactured products. A. W. Rudnick, Jr.

207. Demand for fluid skimmilk. C. H. Berry, E. O. Anderson, D. J. Baker, G. K. Brinegar, L. R. Dowd, and S. Johnson. Storrs Agr. Expt. Sta., *Bull.* 325. 1956.

Using data supplied by the Univ. of Conn. dairy, a carefully controlled experiment concerning relationship of price to consumption was made. On the average, a 1% change in price changed sales 1.14%. The data also indicated that there was substitution of skimmilk for whole milk. A. W. Rudnick, Jr.

208. Review of milk-pricing plans. J. F. Miles, S. C. Agr. Expt. Sta., *Bull.* 438. 1956.

In this comprehensive review of milk-pricing plans, the accepted advantages and disadvantages of each are listed. Some of the plans surveyed are flat price, collective bargaining, classified price, and season price plans. Formulas and government milk-marketing orders also as discussed. A. W. Rudnick, Jr.

FEEDS AND FEEDING

209. A study of techniques for measuring differences in forage quality using dairy cows. M. E. McCULLOUGH. Georgia Agr. Expt. Sta., *Tech. Bull.* (N.S.) 4. 1956.

Data from 60 intake, digestibility, grazing behavior, and persistency of milk production studies on four typical Georgia pastures have been used to compare the value of various techniques in evaluating pasture forage changes. The data show that the time spent grazing was unrelated to forage intake and milk production. The ruminating time was influenced by dry matter intake, digestibility, forage species, and the individual cow. Forage intake was influenced by level of milk production and dry matter intake. Changes in dry matter digestibility apparently reflect changes not only in nutrient availability but also in dry matter intake and ration balance.

A. W. Rudnick, Jr.

HERD MANAGEMENT

210. Financing the dairy system on a central backland farm. C. A. MOORE AND A. C. MAGEE, Texas Agr. Expt Sta., *Bull.* 837. 1956.

This study was made to determine the economic feasibility of changing from cash crop to dairy farming. At 1955 prices it would take from $17,000 to $21,000 to shift to a 36-cow herd producing 9,000 lb. per cow annually. Increased net returns would be $4,768 annually and would be profitable to the dairy man because the additional returns would liquidate the costs in from 4 to 6 yr. A herd producing 7,000 lb. per cow annually would cost $3,600 less than the higher-producing herd, but it would take from 13 to 16 yr. to repay the cost, because annual net income would be increased only $1,526. A. W. Rudnick, Jr.

211. Dairy production in a dry-farming area. M. EVANS, New Mexico Agr. Expt. Sta., *Bull.* 405. 1956.

Selected farms in three eastern New Mexico counties were studied, to determine productivity, income, and costs of small- to medium-sized dairy herds. A. W. Rudnick, Jr.

212. Electric animal-restraining device. R. M. GRAMS. U. S. Patent 2,830,556. 4 claims. February 8, 1957. Offic. Gaz. U. S. Patent Office, **729**, 3: 555. 1958.

Cows are restrained from backward movement in stalls by receiving an electric shock when they touch a contact ring. The current-carrying wire attached to the ring is wound on a revolving drum on the ceiling, which permits raising or lowering the ring to position behind the cow. R. Whitaker.

ICE CREAM

213. Fast-hardening systems. G. M. ANDERSON. Ice Cream Rev., **41**, 9: 26. 1958.

The systems of fast hardening in current use are described and appropriate diagrammatic sketches are presented to illustrate how each system operates.

Hardening time, using a plate or contact hardener maintained at a temperature of −35° F. and with unwrapped cartons, averaged 110 min. for a half-gallon, 80 min. for a square quart, and 50 min. for a flat quart. When using a hardening tunnel with an air temperature of −40° F. and with adequate air turbulence, the hardening time was found to be 105 min., 45 min., and 240 min. for unwrapped half-gallons, pints, and gallon units, respectively. The author suggests that for the most economical operation a temperature of from −30 to −40° F. should be used with tunnel hardening.

The estimated cost of a unit hardener completely installed is $300 per gallon of hourly capacity, or a unit designed to handle 1,000 gal. of product per hour will cost approximately $300,000. W. J. CAULFIELD

MILK AND CREAM

214. Marketing cream in plastic bags. J. L. OLSON, South Dakota Agr. Expt. Sta., *Agr. Econ. Pamphlet* 75. 1956.

A 6-wk. study was made of cream shipped in conventional cans and in plastic bags contained in fiber boxes. Although marked improvement in quality of cream occurred when plastic bags were used, the cost savings were not sufficient to offset the higher price of bag and box. The feasibility of the new system rests entirely on quality improvement. No analysis was made to determine whether or not improved quality was due only to the bags or to the additional care farmers took when learning a new method of handling cream.

A. W. Rudnick, Jr.

215. Preserving milk, Neera, and like perishable liquids. M. A. KAZMI (assignor to Lukas & Assoc.). U. S. Patent 2,829,058. 12 claims. April 1, 1958. Offic. Gaz. U. S. Pat. Office, **729**, 1: 154. 1958.

Bottles of milk or other perishable liquids are placed in a chamber containing a heating liquid which surrounds the bottles up to the neck of the bottle. As the milk is heated to the sterilizing temperature, it expands to fill the bottles exactly. The bottles are then sealed, removed from the chamber, and cooled to leave the product under vacuum and free from air.

R. Whitaker

216. Sterile concentrated milk. J. M. BOYD (assignor to Continental Can Co., Inc.). U. S. Patent 2,827,381. 2 claims. March 18, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 3: 626. 1958.

Concentrated sterile milk is produced by a sequence of processing steps which includes deaeration of raw milk, flash-heating to 120–180° F., homogenizing, flash-heating to 250° for 5 min. or 300° momentarily, aseptically evaporating under reduced pressure at less than 140° F., cooling, and filling into sterile containers. R. Whitaker

MILK SECRETION

217. Studies of the incidence and morphology of accessory glandular tissue in the teat canal of the bovine mammary gland. A. F. Weber, D. S. Wyand, and M. G. Phillips, Coll. Vet. Med., Univ. of Minn., St. Paul. Am. J. Vet. Research, **18**, 69: 761. 1957.

Serial sections were made through the teat canals of 44 teats from udders of 11 dairy cows. One teat contained a bifid teat canal. Two teats had periductal lobules, which were accessory glandules which drained directly into the teat canal about three-fourths the way from the orifice to the rosette of Furstenburg. The alveoli of the glandules showed signs of active secretion. Photomicrographs illustrate the article. E. W. Swanson

PHYSIOLOGY AND ENDOCRINOLOGY

218. Effects of prepartum administration of progesterone to the cow. L. E. McDonald and R. L. Hays, Ill. Agr. Expt. Sta., Urbana. Am. J. Vet. Research, **19**, 70: 97. 1958.

Dairy cows and heifers in the Univ. of Ill. herd were divided into two groups, control and progesterone-treated. Treatment was 500 mg. of progesterone on the 33rd, 23rd, and 13th days before calving and 100 mg. on the 3rd and 2d days before calving. Six of 88 treated cows retained fetal membranes, as did seven of 90 control cows having single births. Observations of rectal temperatures, length of gestation, and subsequent lactation also showed no significant differences between the treated and control groups. The effects of the treatments were in no way harmful and they did not appear to be beneficial in this study. E. W. Swanson

SANITATION AND CLEANSING

219. Cleanability of materials in contact with dairy products. G. L. Hays, J. D. Burroughs, and D. H. Johns, American Can Co., Maywood, Ill. J. Milk and Food Technol., **21**, 3: 68. 1958.

Bacteriological and radioactive tracer methods were used to study the efficiency of removal of soil from the surface of several dairy equipment construction materials. Stainless steel surfaces were soiled with milk products inoculated with *Escherichia coli*. The disks were cleaned with hypochlorite, iodophor, and quaternary ammonium compounds, respectively, at standard recommended concentrations. All disks were sanitized within 3 min.

The results indicate that plastics and the several finishes of stainless steel tested were as readily cleaned as 18-8 stainless steel with 120-grit finish. H. H. Weiser

220. Dairy equipment cleaner. L. F. Bender. U. S. Patent 2,829,657. 6 claims. April 8, 1958. Offic. Gaz. U. S. Pat. Office. **729**, 2:316. 1958.

The teat cups and tubes of a milking machine are placed in a cleaning solution which is caused to flow back and forth through the parts by this device. It is operated by the vacuum supply used for the milker.

R. Whitaker

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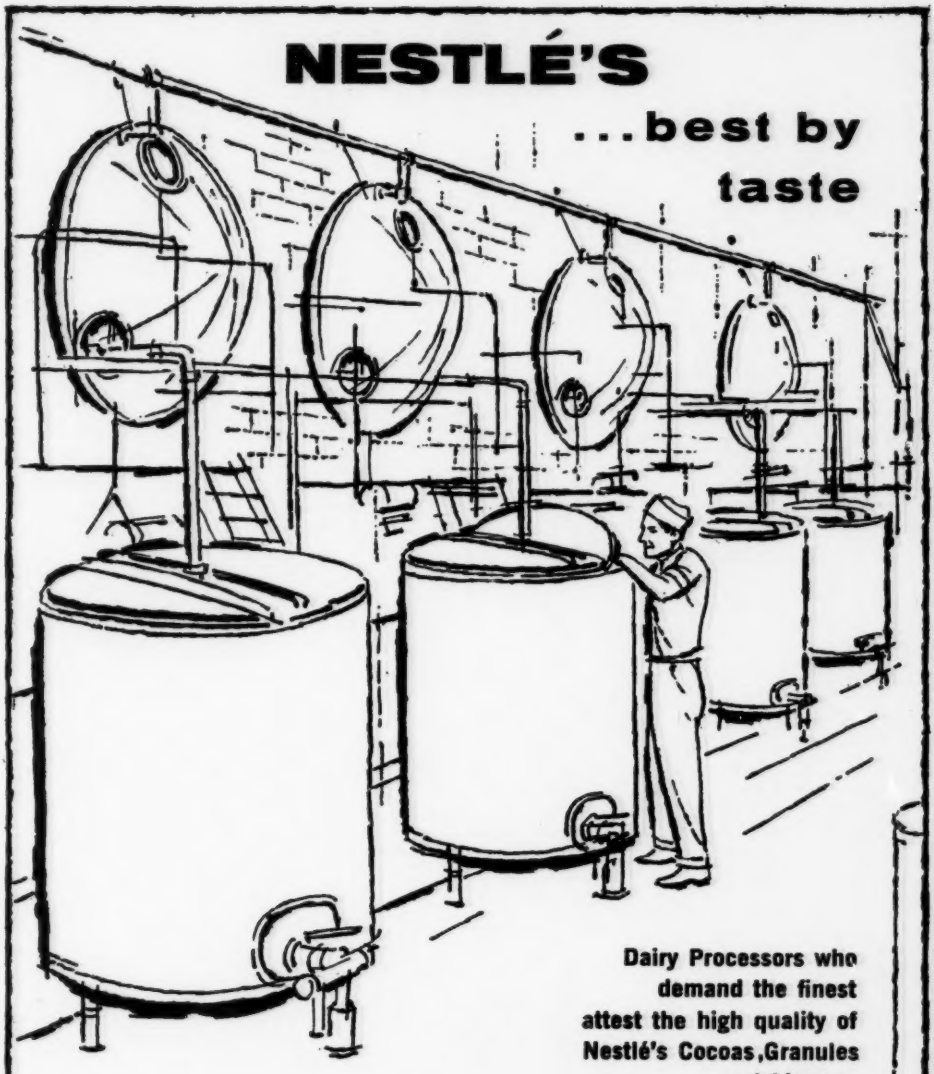

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BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
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